

**UNIVERSITY OF THESSALY**  
**SCHOOL OF HEALTH SCIENCES**



**FACULTY OF VETERINARY SCIENCE**  
**CLINIC OF MEDICINE**

**STUDY ON THE EPIDEMIOLOGY AND THE CLINICAL  
IMPORTANCE OF SELECTED INFECTIOUS,  
PARASITIC AND METABOLIC DISEASES IN CATS  
FROM GREECE**

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**ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ**

**ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ**



**ΤΜΗΜΑ ΚΤΗΝΙΑΤΡΙΚΗΣ**

**ΠΑΘΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ**

**ΜΕΛΕΤΗ ΤΗΣ ΕΠΙΔΗΜΙΟΛΟΓΙΑΣ ΚΑΙ ΤΗΣ  
ΚΛΙΝΙΚΗΣ ΣΗΜΑΣΙΑΣ ΕΠΙΛΕΓΜΕΝΩΝ ΛΟΙΜΩΔΩΝ,  
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**ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ**

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**DOCTORAL THESIS CONDUCTED AT THE CLINIC OF MEDICINE,  
FACULTY OF VETERINARY SCIENCE, SCHOOL OF HEALTH SCIENCES,  
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*Στους γονείς μου*

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## ΣΥΝΤΟΜΟΓΡΑΦΙΕΣ-ABBREVIATIONS

**Α.Π.Θ.:** Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης  
**Γ.Π.Α.:** Γεωπονικό Πανεπιστήμιο Αθηνών  
**Π.Θ.:** Πανεπιστήμιο Θεσσαλίας  
**AG:** albumin/globulin  
**ALP:** alkaline phosphatase  
**ALT:** alanine aminotransferase  
**AST:** aspartate aminotransferase  
**BUN:** blood urea nitrogen  
**CCoV:** canine coronavirus  
**CD:** cluster of differentiation  
**CI:** confidence interval  
**CK:** creatine kinase  
**DNA:** deoxyribonucleic acid  
**EDTA:** ethylenediaminetetraacetic acid  
**ELISA:** enzyme-linked immunosorbent assay  
**FCoV:** feline coronavirus  
**FECV:** feline enteric coronavirus  
**FeLV:** feline leukemia virus  
**FIP:** feline infectious peritonitis  
**FIV:** feline immunodeficiency virus  
**FURTD:** feline upper respiratory tract disease  
**γ-GT:** gamma-glutamyl transferase  
**IFAT:** indirect immunofluorescence antibody testing  
**Ig:** immunoglobulin  
**OR:** odds ratio  
**PCR:** polymerase chain reaction  
**RNA:** ribonucleic acid  
**SARS-CoV:** severe acute respiratory syndrome coronavirus  
**SD:** standard deviation  
**T<sub>3</sub>:** triiodothyronine  
**T<sub>4</sub>:** thyroxine



**TAMU:** Texas A&M University

## ΠΡΟΛΟΓΟΣ

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## PART ONE-REVIEW OF THE LITERATURE

### 1. Introduction

Infectious and parasitic diseases are common in all mammalian species, including domestic cats (*Felis catus*), who are becoming increasingly popular pets in Greece. Although several studies have investigated the prevalence of certain infectious and parasitic diseases in dogs, little is known about the prevalence and epidemiology of specific infectious agents in cats in Greece. This lack of data represents a major drawback for accurate and effective diagnosis, clinical management and prevention of the diseases caused by these infectious agents.

Vector-borne diseases have global impact on the health of domestic cats and are often of zoonotic importance. Due to the fact that fleas, ticks and mosquitoes are common in Greece, vector-borne disease agents such as *Bartonella* spp. and *Mycoplasma* spp., may also be common. In addition, other infectious and parasitic agents, such as feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline coronavirus (FCoV), and *Toxoplasma gondii* are also known to be present in Greece. However, few or no reports exist about their prevalence, epidemiology, and clinical and zoonotic importance of the associated diseases.

Finally, even though feline hyperthyroidism is considered the most common endocrinopathy in cats and, according to epidemiologic studies, the prevalence of the disease is increasing worldwide, no relevant information is available for cats in Greece.

### 2. Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV)

Feline leukemia virus and FIV are retroviruses, causing significant morbidity and mortality in cats.

Although FeLV and FIV are closely related, they differ in their potential to cause disease, because FeLV is more pathogenic than FIV. Both viruses infect domestic cats. In addition, FIV has been isolated from a variety of non-domestic felids, including pumas, lions, leopards and Pallas cats (Soe et al., 1983; Olmsted et al., 1992; Brown et al., 1994; Carpenter et al., 1996; Barr et al., 1997), while FeLV may infect other small felids, including the wild cat *Felis silvestris* and European and Iberian lynxes.

The first description of FeLV was in 1964 in feline lymphoma tissue (Jarrett et al., 1964), while FIV was first isolated in 1986 following the investigation of an immunodeficiency syndrome in a cat colony (Pedersen et al., 1987). Both are members of the *Orthoretrovirinae* subfamily, with FeLV being a  $\gamma$ -retrovirus and FIV being a lentivirus.

Like all retroviruses, FeLV is an enveloped RNA virus that relies on a DNA intermediate for replication. The viral single-stranded RNA is reverse transcribed, by the reverse transcriptase enzyme, into DNA, the so-called provirus, that is usually integrated into the host's cell genome. FeLV exists in several subgroups, the three most important being FeLV-A, FeLV-B and FeLV-C, which are closely related antigenically to each other (Anderson et al., 2000). However, only FeLV-A is contagious to cats, and it passes horizontally, from one cat to another, in nature.

The FIV is associated with a high degree of genetic diversity, leading to the existence of several subtypes, based on envelope gene sequences. However, five major subtypes (A to E) have been recognized worldwide (Sodora et al., 1994), while a new subtype (subtype F) has been recently described in cats from Texas, Portugal, and New Zealand. Most viruses identified so far belong to either subtype A or B.

### **2.1. Transmission and pathogenesis of feline leukemia virus (FeLV)**

Transmission of FeLV occurs primarily via saliva, where virus is found in highest concentrations compared to blood (Hardy et al., 1976). Although transmission may also occur via milk, nasal secretions, feces and urine, their importance in natural transmission is minor (Pacitti et al., 1986). Thus, the major route of transmission is through bite wounds that introduce saliva containing virus, as well as through friendly contacts among cats, such as mutual grooming, sharing water and food dishes, and using common litters. FeLV is transmitted vertically from infected queens to their offspring, leading to embryonic death or viremic kittens which fade rapidly. Iatrogenic transmission may also occur, via contaminated needles, surgical instruments, or blood transfusion, while transmission via fomites is unlikely. Results of a study have shown that cat fleas are potential vectors (*in vitro* isolation of FeLV RNA) but their vectorial role is probably of minor (Vobis et al., 2003).

Initial infection with FeLV usually takes place in the oropharynx and the virus is first found in the local lymphoid tissue. Then, the virus spreads to the periphery via

monocytes and lymphocytes and, during this primary viraemia, it can infect the bone marrow. After bone marrow infection, salivary glands and intestinal linings are infected, leading to virus shedding in large quantities via saliva and feces (Rojko et al., 1982).

Before the development of very sensitive PCR methods, the outcome of infection with FeLV was controversial. Current research has classified the outcomes of FeLV infection as a) abortive infection (comparable to the former “regressor cats”), b) regressive infection (comparable to the former “latent infection”, with or without previous “transient viremia”), c) progressive infection (comparable to the former “persistent viremia”), and d) focal or atypical infection. These outcomes of FeLV infection can be distinguished through testing blood for FeLV p27 antigen, proviral DNA and antibodies. Each outcome depends on the cat’s immune status, the amount of the virus to which the cat was exposed and the duration of contact with a FeLV-shedding cat.

In abortive infections the cat has negative antigen and PCR tests but positive tests for neutralizing antibodies. In these cats, the virus starts to replicate in the local lymphoid tissue of the oropharyngeal area, but replication is terminated by an effective humoral and cell-mediated immune response. This outcome has been linked to a low amount of virus to which the cat has been exposed.

In regressive forms, an effective immune response limits virus replication prior to or at the time of bone marrow infection. Although these cats terminate the viremia, the proviral DNA does exist in bone marrow stem cells. Thus, the cats cannot eliminate completely the virus from their body. Regressor cats remain latently infected for some time, and, rarely, develop FeLV-associated disease such as lymphoma or bone marrow disorders (Pedersen et al., 1977; Flynn et al., 2000; Flynn et al., 2002; Stutzer et al., 2011). Reactivation can occur by stress and/or high doses of corticosteroids or other immunosuppressive drugs (Helfer-Hungerbuehler et al., 2015).

In progressive infection, virus replication occurs, first in the lymphoid tissues, and then in the bone marrow and in mucosal and glandular tissues. These cats are persistently antigenemic, continuously shed the virus, and develop FeLV-associated diseases.

Focal or atypical infections are rare and occur in cats with FeLV infection restricted into certain tissues, such as the spleen, lymph nodes, small intestine,

mammary glands, urinary bladder, and the eyes (Pacitti et al., 1986; Hayes et al., 1989). This persistent atypical local viral replication can lead to intermittent or low-grade production of p24 antigen. Therefore, these cats may have weakly positive or discordant results in antigen tests, or positive and negative results may alternate over time.

## **2.2. Transmission and pathogenesis of feline immunodeficiency virus (FIV)**

Transmission of FIV occurs via saliva or blood, presumably by bites and fight wounds. Vertical or perinatal transmission is rare; however, primary infection of the queen during pregnancy, may result in infection up to 70% of the embryos. In contrast to FeLV, horizontal transmission of FIV between cats, in stable feline populations, rarely occurs. Iatrogenic transmission, via contaminated needles, surgical instruments, or blood transfusions, can also occur, while sexual transmission has not been documented in nature.

Experimental FIV infection is considered to progress in three stages: a transient primary-acute phase, a clinically asymptomatic phase that can last for many years and a terminal “second stage”. In naturally FIV-infected cats these stages may not be clearly distinguishable, while a large proportion of FIV-infected cats may remain free of significant disease or mild clinical signs might not be noticed by their owners (Hayes et al., 1989; Chen et al., 1998; Stutzer et al., 2010; Nesina et al., 2015).

After experimental inoculation, during the acute phase, FIV replicates in dendritic cells, macrophages and CD4<sup>+</sup> T lymphocytes of lymphoid organs including lymph nodes, spleen, thymus, and in organs such as the bone marrow, lung, intestinal tract, brain, and kidney. Moreover, during the acute phase, which lasts 6-8 weeks, FIV can be detected in high concentrations in the blood (by culture and PCR). After peak viremia, an immune response takes place, characterized by the reduction of the circulating viral load and the production of FIV-specific antibodies that occurs 2-4 weeks post-inoculation.

Following the acute phase, the asymptomatic phase begins, which can last for years or be lifelong. During this stage, viremia is absent or in low levels, and for this reason, antigen testing is commonly negative and not recommended in clinical or



epidemiological studies. The infected cat remains free of clinical signs due to successful immune response that inhibits viral replication.

However, at some time point during this stage, a progressive dysfunction of the immune system can occur, and mild or intermittent clinical signs can be seen. In a proportion of infected cats, this can lead to the terminal second stage which is characterized by generalized lymphoid depletion and finally death (Rohn et al., 1994; Tsatsanis et al., 1994; Mendoza et al., 2013).

### **2.3. Epidemiology of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV)**

#### *Prevalence*

The prevalence of these infections varies greatly among countries and regions and depends on the population tested. Studies have reported an overall prevalence of FeLV infection of 2.3-3.3% in North America (Luria et al., 2004; Levy et al., 2006), 0-2.9% in Asia (Nakamura et al., 2000; Maruyama et al., 2003), and 0-15.6% in Europe. A prospective European study on the prevalence of FeLV infection conducted in 32 countries showed an overall prevalence of 2.3% (Studer et al., 2019).

The prevalence of FIV infection has been reported to be 2.5-23% in North America (Little, 2005; Levy et al., 2006), 0-22% in Asia (Miyazawa et al., 1998; Nakamura et al., 2000) and 2.3-11.3% in Europe (Hartmann and Hinze, 1991; Bandecchi et al., 2006).

#### *Risk factors*

Several risk factors for FeLV and FIV infections have been reported and they are only slightly different between these two viruses. Risk factors for both infections include male gender, non-neutering, adulthood, outdoor access, and unhealthy status, while overcrowded conditions and crossbred have been reported as risk factors for FeLV and FIV infection, respectively.

From a behavioral perspective, male cats have been shown to be more aggressive than females, leading to a greater risk of bite wounds and FeLV and/or FIV transmission. Similarly, bite wounds are more common in intact than neutered cats. Outdoor access is also a risk factor for both FeLV- and FIV-seropositivity, since

cats living outdoors or having outdoor access have increased chance to interact with infected cats (Yamamoto et al., 1989; Levy et al., 2006; Hosie et al., 2009). Moreover, older cats are more commonly infected with FeLV and FIV, possibly because they have more time to be exposed to these viruses (Levy et al., 2006; Spada et al., 2012; Liem et al., 2013; Stavisky et al., 2017). Illness is also a risk factor for both retroviral infections. In other words, sick cats tend to be more frequently infected by FeLV and/or FIV than healthy cats, as has been reported in several studies (Little et al., 2009; Burling et al., 2017). Both FeLV and FIV are expected to be more prevalent in sick cats than in healthy ones because both viruses can cause immunosuppression and are associated with many clinical manifestations.

Overcrowded environment is also a risk factor for FeLV, as demonstrated in several studies (Polak et al., 2014; Studer et al., 2019). It is known that transmission of the virus can be either through bite wounds or prolonged close contact with infected cats because of nursing, mutual grooming, and sharing of food, water, and litter pans.

Mixed breed cats are more frequently FIV-infected than pedigree cats (Gleich et al., 2009), mainly because the latter are commonly kept indoors, or their outdoor access is limited. In addition, awareness amongst cat breeders of the risks of FIV infection, leads to frequent testing and, thus, most cat-breeding facilities are kept FIV-free.

#### **2.4. Clinical signs and clinicopathologic abnormalities of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections**

Clinical manifestations of both retrovirus infections are highly variable and depend on viral and host factors, such as the virus subgroup, the cat's age at the time of infection, since older cats are less susceptible (Hoover et al., 1976), and the exposure to secondary pathogens (Podell et al., 1997; Pedersen et al., 2001). For both retroviruses there is a long asymptomatic phase, even in cats with progressive infection, but eventually clinical signs may occur after months to years.

In the acute phase after experimental infection with FIV, which can last several days to months, clinical signs are mild and include fever, generalized lymph node enlargement (Obert and Hoover, 2000), enteritis, stomatitis, dermatitis, conjunctivitis, and upper respiratory tract disease.

After a long asymptomatic phase, a wide range of disorders are associated with FeLV or FIV infections, including tumors, blood dyscrasias, neurologic dysfunction, immune-mediated diseases, immunosuppression with secondary opportunistic infections, gingivostomatitis, reproductive disorders and fading kitten syndrome (Hoover and Mullins, 1991; Tenorio et al., 1991; Ryan et al., 2005; Hartmann, 2011). The pathomechanism of these disorders is different for each virus. It is also worth mentioning that FeLV potentially causes severe clinical syndromes and progressive infection is associated with a decrease in life expectancy, since 70% to 90% of progressively infected cats will die within 18 months to 3 years (Hardy et al., 1976). On the contrary, most naturally FIV-infected cats do not present severe clinical syndromes, with most of the latter being potentially treatable.

The most common tumors associated with FeLV infection are lymphoma and leukemias, less often other hematopoietic tumors, and rarely non-hematopoietic malignancies, including fibrosarcoma, osteochondroma and neuroblastoma.

The most common tumors associated with FIV infection are lymphoma and leukemia and uncommonly fibrosarcoma, squamous cell carcinoma and mast cell tumor. FIV-infected cats mostly develop B-cell lymphomas, and they have 5-fold increased risk of lymphoma or leukemia compared to non-infected cats (Poli et al., 1994; Callanan et al., 1996). In contrast to FeLV, it is proposed that FIV causes oncogenesis indirectly (Beatty et al., 1998; Wang et al., 2001) due to chronic B-cell hyperplasia or decreased cell-mediated immune surveillance.

Due to immunosuppression, FeLV- and FIV-infected cats are more susceptible to secondary infections and/or to infection by agents to which they would normally be resistant, such as *Salmonella* spp. However, the immunosuppression is more severe for FeLV and infected cats often have concurrent bacterial, viral, protozoal, and/or fungal diseases (Cotter, 1991; Powers et al., 2018), including feline upper respiratory tract disease-FURTD (O'Connor et al., 1991; Barrs et al., 2000; Crawford et al., 2001; Sykes et al., 2007; Bauer et al., 2008; Macieira et al., 2008; Gentilini et al., 2009; Burling et al., 2017; Studer et al., 2019). On the other hand, bacterial skin diseases that have been associated with FIV include folliculitis, superficial spreading pyoderma, abscesses, paronychia, and otitis. Fungal, viral, and parasitic infections, such as dermatophytosis, dermatophyte pseudomycetoma and infections with *Candida*, *Malassezia*, *Demodex cati*, and cow-poxvirus, have also been reported in FIV-positive cats. In addition, idiopathic lymphocytic mural folliculitis, plasma cell

pododermatitis, and generalized miliary dermatitis with alopecia and scaling have been associated with FIV.

Gingivostomatitis is very common in retrovirus-infected cats and is one of the most typical clinical findings of FIV infection, being present in up to 50% of the cases (Hofmann-Lehmann et al., 1995; Knotek et al., 1999; Madhu Ravi, 2010; Ravi et al., 2010; Kornya et al., 2014; Beczkowski et al., 2015). Gingivostomatitis has been attributed to an aberrant immune response against chronic antigenic stimulation or to immune dysregulation (Tenorio et al., 1991).

Immune stimulation caused by retroviruses, may lead to immune-mediated diseases, which more frequently develop in FIV- than FeLV-infected cats. These diseases are caused by loss of T-suppressor cell activity and the formation of antigen-antibody immune complexes, and include immune-mediated hemolytic anemia, glomerulonephritis, uveitis, and polyarthritis (Anderson et al., 1971; Brightman et al., 1991).

Gradually deteriorating neurologic dysfunction is one of the few syndromes that are directly caused by FeLV and FIV. In FeLV-infected cats, most neurologic signs are caused by lymphoma or lymphocytic infiltrations in the central nervous system and the main clinical manifestations include anisocoria, mydriasis, central blindness, Horner's syndrome, urinary incontinence, hyperesthesia, paresis, and paralysis (Carmichael et al., 2002; Forman et al., 2009). Similar signs are present in about 5% of symptomatic FIV-infected cats and they seem to be strain-dependent (Power et al., 1998). They include behavioral changes, disturbed sleep patterns, twitching movements of the face and tongue, compulsive roaming, delayed righting and pupillary reflexes, anisocoria, loss of bladder and rectal control, seizures, ataxia, nystagmus, and intention tremors (Prospero-Garcia et al., 1994; Gunn-Moore et al., 1996; Phillips et al., 1996; Steigerwald et al., 1999).

Reproductive disorders attributed to FeLV include abortion, fetal resorption, and neonatal death. Abortion usually occurs in the late stages of gestation and sometimes is followed by bacterial endometritis, especially if there is concurrent neutropenia. Fading kitten syndrome may develop when kittens are born by infected queens. Thymic atrophy is observed in these kittens, resulting in severe immunosuppression, wasting and early death usually within the first 2 weeks of their life (Dunham and Graham, 2008).

In some FeLV-infected cats a diagnosis of benign peripheral lymphadenopathy has been reported. This sign should be interpreted with caution since it must be differentiated from multicentric lymphoma (Moore et al., 1986).

Both FeLV and FIV infections are associated with a variety of clinicopathologic abnormalities. Blood dyscrasias can occur due to bone marrow suppression and they are more common and more severe in FeLV-infected cats. Hematologic disorders related to FeLV infection include anemia, leukocyte and platelet abnormalities and pancytopenia.

Overall, FeLV-infected cats may develop anemia, usually non-regenerative, through various pathomechanisms. Regenerative anemia is uncommon and may be associated with blood loss (e.g., due to thrombocytopenia) or hemolysis (e.g., concurrent infection by hemoplasmas or immune-mediated hemolytic anemia). Nonregenerative anemia may occur due to bone marrow aplasia, myelophthisis, chronic disease, myelodysplasia, myelofibrosis, and nutritional deficiencies (Shelton and Linenberger, 1995; Shimoda et al., 2000; Hisasue et al., 2001; 2006). Anemia of chronic disease (e.g., concurrent infections or neoplasia), occurs because of excessive inflammatory cytokine production and subsequent abnormal iron utilization and reduced red blood cell survival. Pure red cell aplasia is a severe nonregenerative anemia which is typically due to FeLV subtype C, because this virus interacts and downregulates the cell-surface receptor that functions as heme exporter, resulting in cell death (Tailor et al., 1999; Quigley et al., 2000; George et al., 2002). Nevertheless, the type of this nonregenerative anemia is rare. Thrombocytopenia may occur, due to immune-mediated destruction of platelets or bone marrow suppression. In addition, giant platelets, thrombocytosis, and shorter platelet lifespan, have been observed in some progressively FeLV-infected cats. Neutropenia is present in some cats and can be caused by immune-mediated mechanisms and/or myelosuppression (Brown and Rogers, 2001; Gleich and Hartmann, 2009; Stutzer et al., 2010). Lymphopenia, because of lymphocyte destruction from direct replication of FeLV, may also occur and it is characterized by loss of CD4<sup>+</sup> helper T cells and inverted CD4/CD8 ratio (as typically seen in FIV infection).

In contrast to FeLV, FIV-associated cytopenias are rather uncommon. The most common hematological abnormality is neutropenia, which is present in about 25% of infected cats. Neutropenia may occur either in the acute phase of infection (and resolve in the asymptomatic phase), or in the later phase of infection when the

cat develops clinical signs. During this phase, patients may also develop other cytopenias including lymphopenia and anemia, which in most cases is mild.

Abnormalities found on serum biochemistry in FeLV and FIV-infected cats are typically mild and, in most cases, reflect concurrent diseases. Nevertheless, FIV-infected cats have a significantly increased risk of hyperglobulinemia (Hofmann-Lehmann et al., 1997; Miro et al., 2007; Gleich and Hartmann, 2009; Stutzer et al., 2010). This is a direct consequence of the virus that triggers polyclonal B cell activation and immunoglobulin production, and has been documented in both natural and experimental infections (Flynn et al., 1994). Less commonly, azotemia has been reported in FIV-infected cats in the absence of other detectable causes of kidney disease (Poli et al., 1993). In a long-term study of experimentally FeLV-infected cats, urea concentrations and serum activities of AST were significantly higher compared to non-infected controls (Soe et al., 1983).

### **3. Feline coronavirus (FCoV)**

Feline coronavirus (FCoV) is a large, spherical, enveloped, single-stranded, positive-sense, RNA virus classified in the order *Nidovirales*; family *Coronaviridae*; genus *Alphacoronavirus*; species *Alphacoronavirus* 1. It is a highly contagious and ubiquitous virus of domestic cats and wild felids and is extremely common in crowded environments. FCoV causes enteric infection and sporadically leads to a fatal, systemic disease, named feline infectious peritonitis (FIP). With the discovery of the severe acute respiratory syndrome coronavirus (SARS-COV) that commonly infects bats, and apparently jumped from civets and raccoon to humans causing the recent and ongoing pandemic, the suspicion that coronaviruses may be responsible for zoonoses has been reinforced. However, FCoV is not infectious for other species (including humans) and is different from the coronavirus which causes COVID-19 in people which is a *Betacoronavirus*.

FCoV strains are subdivided into two distinct biotypes; feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV), which are both divided into two serotypes, I and II. Serotype I FCoV includes unique feline strains, while serotype II appears to have arisen from recombination between type I FCoV and canine coronavirus (CCoV). Although serotype I is most prevalent worldwide, both serotypes I and II can cause FIP.

### **3.1. Transmission**

Transmission of FCoV occurs primarily via the fecal-oral route. The major source of infection is the litter box, and the transmission results from ingestion of infected feces, by paw grooming during litter tray use, or by eating contaminated food. Transplacental transmission and transmission via saliva may occur, but their importance is minor (Addie and Jarrett, 1990, 2001). The virus can persist on fomites for 3-7 weeks (Hartmann, 2005; Pedersen, 2009; Kipar et al., 2010).

### **3.2. Pathogenesis**

Regarding the two biotypes of FCoV, FECV mainly replicates in the intestinal epithelium, causing occasionally mild enteritis, whereas FIPV replicates in the monocytes and macrophages, causing fatal disease. Since all FCoV can be found and replicate systemically (Addie et al., 2009), it is now accepted that these viruses are not different species, but they represent virulence variants of the same virus. Thus, it is suggested to call both biotypes FCoV, including "the less virulent FCoV" and "the FIP-associated FCoV". The most widely accepted hypothesis of developing FIP is the internal mutation theory. Infection with FCoV initially occurs in intestinal epithelial cells, and subsequently, in an unpredictable proportion of cats, the virus mutates and acquires the ability to enter and efficiently replicate in monocytes and macrophages, which allows for the severe disease. The mutations causing this critical change in virulence have been ascribed to numerous genes (spike gene and accessory genes; 3c, 7a and 7b).

Although FCoV infection is typically asymptomatic, mild diarrhea may rarely occur due to replication of the virus in enterocytes (Sabshin et al., 2012; Desmarests et al., 2013). On the contrary, the clinical signs of FIPV infection vary considerably. Two clinical forms of FIP are well-documented: a "wet" or effusive form, characterized by vasculitis and polyserositis, and a "dry" or non-effusive form, characterized by pyogranulomatous lesions in several organs (Kipar et al., 2005). Many cats may, in fact, may share features of both forms of the disease.

### **3.3. Epidemiology**

#### *Prevalence*

FCoV infection has been reported worldwide and around 40% of domestic cats are infected. Since FCoV is highly contagious, the prevalence of seropositivity may be up to 90% in crowded environments, like catteries, shelters, and multi-cat households (Addie and Jarrett, 1992b; Addie, 2000). However, only 5% to 12% of infected cats develop FIP (Addie and Jarrett, 1992a; Addie and Jarrett, 1992b; Addie et al., 1995). In addition, studies have reported an overall seroprevalence of FCoV of 0-84% in Europe (Sparkes et al., 1992; Herrewegh et al., 1997; Chatzis et al., 2014), 0-71.6% in America (Pedersen, 1976; Levy et al., 2008), 6.6-28.2% in Asia (An et al., 2011; Wang et al., 2014), 18-40% in Australia (Jones and Hogg, 1974; Watson et al., 1974) and 35% in South Africa (Bland van den Berg and Botha, 1977).

### *Risk factors*

The most well-documented risk factor for FCoV-seropositivity is living in multi-cat environments, such as breeding catteries and shelters. In addition, not only the number of the cats, but also their population density, and the length of time they stay in these environments are associated with the prevalence of FCoV infection. In a study from the United Kingdom, it was reported that cats originating from multi-cat households were approximately two times more likely to be FCoV-seropositive compared to cats from single-cat households (Cave et al., 2004). Similarly, in a preliminary study on the epidemiology of FCoV in cats from Turkey, the reported seroprevalence among cats from multi-cat environments was 62% versus 4% among single-cat households (Pratelli et al., 2009). A study of cats relinquished to a United Kingdom rescue charity indicated that cats spent more than 60 days in a shelter were five times more likely to be FCoV-seropositive (Cave et al., 2004). On the contrary, stray or feral cats are less likely to be FCoV-seropositive compared to pet cats, sheltered cats and cats living in catteries, likely due to lower population densities and because the former have a larger territory and they bury their feces, minimizing the fecal-oral FCoV transmission.

In addition to the risk factors mentioned above, male gender, young age, and purebred have also been suggested as risk factors for developing FIP (Pedersen, 1976; Rohrbach et al., 2001; Cave et al., 2004; Pesteanu-Somogyi et al., 2006; Sharif et al., 2009). In two studies, conducted in Taiwan and North Carolina, 88% and 67% of FIP-confirmed cases, respectively, were less than 2 years old (Rohrbach et al., 2001; Tsai et al., 2011) and, in general, 70% of FIP cases occur in cats younger than 1 year



(Hartmann, 2005). Several breeds have been found to be more susceptible to the disease, including Abyssinian, Bengal, Birman, British Shorthair, Devon Rex, Himalayan, and Ragdoll (Pesteanu-Somogyi et al., 2006; Worthing et al., 2012). In addition, cats with high titers of antibodies against FCoV have a greater risk of developing FIP (Poland et al., 1996; Foley et al., 1997; Pedersen, 2009). Stress experienced by FCoV-infected cats, such as after surgery or moving to a new environment, may precipitate the development of FIP (Riemer et al., 2016) and the same is true for immunosuppressive conditions, such as co-infection with FeLV and FIV (Cotter et al., 1975; Hardy et al., 1976; Foley et al., 1997).

#### **4. *Bartonella* spp.**

Bartonellosis is a vector-borne disease with global impact on the health of domestic cats and of zoonotic importance (Boulouis et al., 2005; dos Santos et al., 2008; Lin et al., 2011). *Bartonella* species are small, Gram-negative intracellular bacteria and cats are the main reservoir of *Bartonella henselae*, *B. clarridgeiae* and *B. Koehlerae*, and accidental hosts of *B. quintana*, *B. bovis* and *B. vinsonii* subsp. *berkhoffii*. The most common species infecting cats is *B. henselae*, the main causative agent of cat scratch disease (CSD) in humans (Boulouis et al., 2005), followed by *B. clarridgeiae*. Cat scratch disease is characterized by regional lymphadenopathy and prolonged or relapsing fever, but immunocompromised people may additionally present encephalopathy, osteomyelitis, retinitis, endocarditis, bacillary angiomatosis and parenchymal bacillary peliosis.

##### **4.1. Transmission and pathogenesis**

*B. henselae* is naturally transmitted among cats by the cat flea *Ctenocephalides felis* (Bouhsira et al., 2013). Adult *C. felis* can become infected and support replication of *B. henselae* following ingestion of a blood meal from an infected cat. Experimentally infected adult fleas remain infectious for their entire life, starting from 24 h after ingestion of the blood meal, and the bacteria can be cultured from flea feces up to 9 days after they were fed infected blood (Higgins et al., 1996). The primary mode of transmission to cats is by contamination of open wounds with feces of infected fleas, while other modes could be the ingestion of infected fleas or flea feces and fighting with other cats (Bouhsira et al., 2013). It has been suggested

that ticks may also have a vectorial role, since transstadial transmission of *B. henselae* has been demonstrated in *Ixodes ricinus* (Cotte et al., 2008). Non-vectorial transmission may occur through blood transfusions, since experimentally infected cats develop chronic and persistent bacteremia (Kordick and Breitschwerdt, 1997).

In the infected cat, *Bartonella* inhabits erythrocytes and vascular endothelial cells. Following infection, chronic and recurrent bacteremia occurs mostly in young cats, under the age of two years. Cats experimentally infected by *B. henselae* and *B. clarridgeiae* presented bacteriaemia for as long as 454 days post-infection (Kordick et al., 1999), and bacteremia was intermittent, occurring at irregular intervals ranging between 1 and 4.5 months. Prolonged (for at least 3 years) recurrent bacteremia has also been demonstrated in naturally infected cats, but this was more likely due to their reinfection. The main factor accounting for the persistent bacteremia is the ability of the organism to avoid elimination by the immune system, via intracellular location, frequent genetic rearrangements, and alteration of outer membrane proteins. As the host-adapted reservoir of *Bartonella*, infection of cats usually has minimal health consequences.

## 4.2. Epidemiology

### *Prevalence*

The prevalence of *Bartonella* spp. infection varies greatly among countries and regions and depends, not only on the feline population tested, but also on the environmental conditions, with highest figures in areas that are favorable for arthropod vectors, mainly fleas. The seroprevalence has been reported to be 0-68% in Europe (Bergh et al., 2002; Mietze et al., 2011), 0-85.2% in the USA (Jameson et al., 1995; Levy et al., 2008), 37% in Australia (Barrs et al., 2010), and 11-59% in Africa (Barrs et al., 2010; Al-Kappany et al., 2011; Tiao et al., 2013). For comparison, epidemiological studies suggest that the overall prevalence of active infection is typically less than 15% (Solano-Gallego et al., 2006; Ishak et al., 2007; Kamrani et al., 2008; Tabar et al., 2008; Gutiérrez et al., 2013; Maia et al., 2014; Silaghi et al., 2014; Otranto et al., 2017), but higher rates have also been reported, such as 67% in Portugal (Alves et al., 2009).

### *Risk factors*

The most well-documented risk factor for *Bartonella* spp. infection is flea infestation (Chomel et al., 1996; Foil et al., 1998; Guptill et al., 2004). In addition, younger cats, especially those under 2 years, are more likely to present bacteremia compared to older cats, while the latter appear to be more likely to become seropositive compared to former (Zangwill et al., 1993; Koehler et al., 1994; Chomel et al., 1995; Jameson et al., 1995; Ueno et al., 1995; Breitschwerdt and Kordick, 2000). Cats with outdoor access are more likely to become infected, due to increased access to flea-infested environment (Chomel et al., 1995; Branley et al., 1996; Chomel et al., 1999; Finkelstein et al., 2002; Guptill et al., 2004; Nutter et al., 2004). Similarly, multi-cat household is a risk factor for *Bartonella* infection due to the increased number of potential hosts (other infected cats) and vectors (Gurfield et al., 2001).

### **4.3. Clinical signs and clinicopathologic abnormalities**

Cats naturally infected with *Bartonella* spp. may have chronic bacteremia but no clinical signs; rarely, they develop endocarditis, myocarditis, osteomyelitis, polyarthritis, and uveitis (Guptill et al., 1997; Kordick et al., 1999). In addition, it has been suggested that *Bartonella* infection could play a role in chronic gingivostomatitis, presumably due to an immune response to chronic antigenic stimulation. However, conflicting data exist regarding the association between gingivostomatitis and *Bartonella* spp. bacteremia and/or seropositivity. In two studies of naturally infected cats, an association between seropositivity and stomatitis was suggested (Ueno et al., 1996; Glaus et al., 1997), while, in another study, an association was found between *Bartonella* spp. bacteremia and oral disease, but not between seropositivity and oral disease (Sykes et al., 2010). Conversely, results of a study in cat shelters, indicated an association between seropositivity, but not bacteremia, and oral lesions (Namekata et al., 2010). The association between gingivostomatitis and *Bartonella* spp. infection requires further investigation.

Most cats experimentally infected with *Bartonella* spp. do not show clinical signs. Occasionally, when signs occur, they are mild and vary depending on the inoculated strain of *B. henselae*. The reported clinical signs include self-limited lethargy and anorexia (Kordick et al., 1999; Namekata et al., 2010), localized or

generalized peripheral lymphadenomegaly, mild neurologic signs, and reproductive failure.

Laboratory abnormalities in naturally infected cats are limited to mild anemia, leukocytosis, eosinophilia, and typically polyclonal hyperglobulinemia due to the prolonged bacteremia (Chomel et al., 2003; Ishak et al., 2007; Whittemore et al., 2012).

## **5. *Mycoplasma* spp.**

Hemoplasmas (hemotropic mycoplasmas) are organisms that infect red blood cells. They can result in hemolytic anemia in a wide variety of mammals, including domestic cats. Many of them are now classified within the genus *Mycoplasma* of the *Mycoplasmataceae* family (Neimark et al., 2001; Messick et al., 2002; Neimark et al., 2004). However, results of a recent study suggest that although hemoplasmas belong to this family, they may comprise their own separate genus (Hicks et al., 2014). The three main species that are known to infect cats, alone or in combination, include, *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum* and *Candidatus Mycoplasma turicensis*. Moreover, a fourth species, *Candidatus Mycoplasma haematoparvum*-like has recently been reported in cats (Martinez-Diaz et al., 2013), but its clinical importance remains unclear (Sykes et al., 2007; Martinez-Diaz et al., 2013).

### **5.1. Transmission**

Although the natural mode of transmission of the infection among cats is not clear, arthropod vectors, including *C. felis*, and interaction with other infected cats have been proposed (Sykes et al., 2007; Museux et al., 2009; Sykes et al., 2010). In particular, *C. felis* has been implicated in feline hemoplasma transmission (Woods et al., 2003; Lappin et al., 2004) and, in a study, cats experimentally infected with *M. haemominutum* transferred the organism to fleas (Woods et al., 2005). Ticks have also been proposed as potential vectors, because feline hemoplasma DNA has been amplified from ticks parasitizing cats (Duplan et al., 2018). However, concrete evidence for their role in the natural transmission of feline hemoplasmas does not exist. It has been suggested that cat fights are involved in transmission and that cat-bite abscesses are the most frequent disorder preceding infection with hemoplasma by

a few weeks. Furthermore, the association between hemoplasma infection and male gender and/or FIV infection, demonstrated in some studies, could also imply that cat fights are a potential route of transmission. Another possible means of transmission is blood transfusion (Pennisi et al., 2015).

## 5.2. Pathogenesis

*M. haemofelis* is the most pathogenic feline hemoplasma species. Acute infection may result in severe, and sometimes life-threatening, hemolytic anemia, but, in some cats, the anemia is mild. The acute disease may occur in immunocompetent cats, and irrespectively of the age, although younger cats are more likely to develop clinical illness (Tasker, 2010). Based on studies of experimentally infected cats, clinical signs occur 2-34 days post-infection and anemia lasts from around 18 up to 30 days. In most cases, the anemia is followed by a significant regenerative response with marked reticulocytosis.

*Ca. M. haemominutum* infection is rarely the causative agent of anemia in the absence of co-infection with another hemoplasma species or of immune compromise. However, rare cases of mild to moderate anemia have been reported in the absence of concurrent infections (Reynolds and Lappin, 2007; Weingart et al., 2016). In general, it is possible that different strains of each of the feline hemoplasma species exist, and that these strains vary in pathogenicity.

Like *Ca. M. haemominutum*, the pathogenicity of *Ca. M. turicensis* is supposed to be mild and that concurrent infections should co-exist to result in hemoplasmosis, although hemolytic anemia has been reported (Willi et al., 2005).

Long-term carrier status can occur with all feline hemoplasmas. Cats with latent *M. haemofelis* infections (carriers) occasionally have low number of organisms visible in blood smears. Their HCT may be normal or slightly to moderately decreased, but typically not lower than 20%. Carrier cats do not show clinical signs, but reactivation can occur and result in disease (Harvey and Gaskin, 1978).

### 5.3. Epidemiology

#### *Prevalence*

The prevalence of hemoplasma infection varies greatly among countries and regions and depends on the feline population tested. Countries with warmer climates appear to have higher prevalence rates.

In general, on the basis of studies that have investigated infection by all three hemoplasma species by PCR, *Ca. M. haemominutum* has been found to be the most common species, with prevalence ranging from 0% to 46.7% (Bauer et al., 2008; Kamrani et al., 2008; Levy et al., 2008; Gentilini et al., 2009; Barrs et al., 2010; Tanahara et al., 2010; Lobetti and Lappin, 2012). *M. haemofelis* and *Ca. M. turicensis* follow with median prevalence of 4.8% and 2%, respectively.

All three hemoplasma species have been described worldwide, sometimes in combined infections. The overall PCR-based prevalence of feline infections with hemoplasmas, has been reported at 32.1% in South Africa (Lobetti and Tasker, 2004), 27.2% in Australia (Tasker et al., 2004), 26.4% in Asia (Tanahara et al., 2010), 12-27% in the USA (Sykes et al., 2008) and 4-43% in Europe (Mifsud et al., 2020).

#### *Risk factors*

In several studies, male gender has been reported as a risk factor for hemoplasma species infection (Harrus et al., 2002; Willi et al., 2006; Sykes et al., 2008). From a behavioral perspective, male cats have been shown to be more aggressive than females, leading to a greater risk of bite wounds and hemoplasma transmission. Similarly, outdoor access and cat bite abscesses have been suggested as risk factors (Willi et al., 2006). Cats living outdoors or having outdoor access have an increased chance of aggressive interactions with hemoplasma-infected cats and of exposure to fleas. Non-pedigree cats are more likely to become infected compared to purebred cats (Jenkins et al., 2013). The results of several studies indicate that older cats are more likely to be infected compared to younger cats (Tasker et al., 2003; Willi et al., 2006). A possible explanation of this association could be that older cats have higher chance of acquiring chronic subclinical infections over their lifetime. Finally, some studies have suggested that FIV is a risk factor for hemoplasma infection (Macieira et al., 2008; Gentilini et al., 2009).

As already mentioned, *C. felis* has been implicated in the transmission of feline hemoplasmas, despite the limited evidence for transmission by fleas under experimental or natural conditions (Woods et al., 2006). However, regular use of ectoparasiticides has been associated with decreased risk of hemoplasma infection (Tasker et al., 2018).

#### **5.4. Clinical signs and clinicopathologic abnormalities**

The clinical signs of hemoplasma infections are variable and depend on the stage of the infection, the host response to the organism, the health status of the host, and the hemoplasma species involved. Many of the clinical signs of hemoplasmosis result from anemia or the underlying immune-mediated processes. Naturally infected cats may have asymptomatic chronic bacteremia, or they may develop fever, anorexia, weight loss, dehydration and/or splenomegaly (Harrus et al., 2002; Tasker, 2010). Lethargy and weakness have also been reported. In a recent retrospective study, the prevalence of hemoplasma infection in 106 cats with pyrexia was 7.5% (Spencer et al., 2017), and in a study of 22 anemic cats naturally infected with hemoplasma species, 10% of them had fever (Weingart et al., 2016). In experimentally infected cats, only those infected with *Ca. M. haemominutum* showed mild or no clinical signs, with occasional fever (Westfall et al., 2001).

Laboratory abnormalities include mild to severe hemolytic anemia, leukopenia, lymphopenia, eosinopenia, monocytosis, hypergammaglobulinemia, hyperbilirubinemia, and increased activity of alanine aminotransferase (Kewish et al., 2004; Raimundo et al., 2016).

#### **6. *Toxoplasma gondii***

Toxoplasmosis is one of the most important protozoan diseases with a global impact on the health of domestic cats and is also of zoonotic importance (Tenter et al., 2000). The causative agent is *Toxoplasma gondii*, which infects virtually all species of warm-blooded animals. Domestic cats and other felids are the definitive hosts that excrete oocysts (Su et al., 2003).

## 6.1. Transmission

The main modes of transmission, in both intermediate and definitive hosts, are congenital infection through the placenta, ingestion of oocyst-contaminated food or water and ingestion of infected raw tissue, with the latter being the primary source of infection in cats (Hill and Dubey, 2002). Other minor modes of transmission include blood transfusion, lactation and transplantation of tissues and organs (Bernsteen et al., 1999).

## 6.2. Pathogenesis

During the life cycle of *T. gondii*, three developmental stages can infect cells: a) tachyzoite (a form of rapid multiplication that is found in acute infections), b) bradyzoite (a form of slow multiplication that is characteristic of chronic infection terminating to tissue cyst formation), and c) sporozoite, which is produced only in the definitive host during the sexual reproduction and is released as oocysts in the feces.

The parasite has two different life cycles, the entero-epithelial that occurs only in the definitive feline host, and the extra-intestinal which occurs in all hosts. Most cats are infected by ingesting intermediated hosts. Following ingestion, bradyzoites are released in the stomach and intestine of the cat from the tissue cysts of the prey. The sexual, entero-epithelial cycle follows and terminates in the shedding of oocysts to the environment through feces. Cats can shed oocysts for only 3-10 days after infection by tissue cysts-bradyzoites (97% of cases), or for up 18 days in the rare case that cats become infected by ingesting oocysts or tachyzoites (Dubey, 1996, 1997, 2002). Once passed to the environment, sporulation occurs in 1-5 days and the sporulated (infectious) oocysts can survive for months to years. After the primary infection of a cat, subsequent re-infections do not usually result in oocyst shedding, and even if this occurs, the load of shed oocysts is low.

The extra-intestinal life cycle of *T. gondii* may occur after the ingestion of tissue cysts or oocysts. After the exposure, sporozoites excyst in the lumen and become tachyzoites by an asexual process. Tachyzoites multiply intracellularly in almost in any cell of the body and eventually encyst. The ensuing tissue cysts contain numerous bradyzoites.



Feline infection with *T. gondii* is usually subclinical, although vertical toxoplasmosis or infection of immunocompromised cats may cause severe disease, and even death (Dubey et al., 2020).

### 6.3. Epidemiology

#### *Prevalence*

The global prevalence of *T. gondii* seropositivity, based on meta-analyses, has been reported to be 35% and 32.9% in cats and humans, respectively (Bigna et al., 2020; Montazeri et al., 2020). Several studies have examined the seroprevalence of *T. gondii* in cats in various countries, and, based on the results, a wide geographical variation is evident. In Europe, seroprevalence has been reported to be 41-60.8% in Northern Europe (Must et al., 2015; Saevik et al., 2015), 10-84.7% in Southern Europe (Millan et al., 2009; Miro et al., 2011), 19.2-65.5% in Western Europe (Bennett et al., 2011; Afonso et al., 2013), 14.7-39.3% in Eastern Europe (Pavlova et al., 2016; Shuralev et al., 2018) and 47-81.3% in Central Europe (Sroka and Szymańska, 2012; Shuralev et al., 2018). In America, studies have reported an overall seroprevalence of 1.1-100% in North America (Bevins et al., 2012; Jimenez-Coello et al., 2013), 0-82.8% in South America and 25% in Central America (Furtado et al., 2015; Teixeira et al., 2016; Rengifo-Herrera et al., 2017). In addition, the reported seroprevalence in North Africa varies from 50% to 97.5%, in West Africa from 4.4% to 36.2% and it is reported to be 3.9% in South Africa (Al-Kappany et al., 2010; Kamani et al., 2010; Ayinmode et al., 2017; Lopes et al., 2017; Yekkour et al., 2017), while in Asia ranges between 2.2% and 82.8% (Hong et al., 2013; Asgari et al., 2018).

#### *Risk factors*

The greatest risk factor for *T. gondii* seropositivity is hunting and ingestion of tissue cysts in prey species, including rodents and birds. Also, ingestion of mechanical vectors such as cockroaches and earthworms has been suggested as a possible mode of infection. Outdoor access is a risk factor for seropositivity, and this is obviously associated with the opportunity of an outdoor cat to hunt the intermediate hosts (Miro et al., 2004; Hornok et al., 2008; Opsteegh et al., 2012; Munhoz et al., 2017; Schreiber et al., 2021). Several studies have suggested that older cats are more

commonly infected, possibly because the chances of exposure to the parasite increase over time (Gauss et al., 2003; Maruyama et al., 2003; Vollaire et al., 2005). The results of studies investigated the breed as risk factor are controversial. In a study from Estonia, purebred was a risk factor for seropositivity (Must et al., 2015) and in another study from Finland, Birman and Ocicats were more likely to be seropositive compared to five other breeds (Jokelainen et al., 2012; Must et al., 2017).

## **7. Feline hyperthyroidism**

Hyperthyroidism is a multi-systemic disorder resulting from excessive circulating concentrations of the thyroid hormones, thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>). It occurs mainly in middle-aged and old-aged cats, with a reported age range of 2-25 years and a median age at the time of diagnosis of 11.5-15.1 years (Milner et al., 2006; Williams et al., 2010; Peterson, 2013; Peterson and Broome, 2015; Puig et al., 2015; Grossi et al., 2019). Benign adenomas or adenomatous hyperplasia affecting one (30%) or both lobes (70%) of the thyroid gland are the most common causes of the disease, accounting for 97-99% of the cases, while thyroid carcinomas are rare (Turrel et al., 1988; Peterson et al., 1994; Naan et al., 2006). To date, the underlying etiology responsible for the thyroid changes has remained unclear and is likely to be multifactorial including genetic, nutritional and environmental influences.

### **7.1. Epidemiology**

#### *Prevalence*

Hyperthyroidism is the most common endocrinopathy of cats, with 1-4% prevalence in the general feline population and at least 6% in cats older than 9 years (Milner et al., 2006; Trepanier, 2006; Wakeling et al., 2011). Since its initial description (Holzworth et al., 1980), the prevalence (or the frequency of diagnosis) of the disease has been steadily increased worldwide (McLean et al., 2017) and there is strong evidence that it differs among different geographical areas (Scarlett et al., 1988).

Depending on the selection criteria (mainly the age) of the cats enrolled in each study, reported prevalence rates vary widely, from 0.2% to 21.1% in Europe,

from 0.1 to 2% in North America, and from 3.9% to 8.9% in Asia (Edinboro et al., 2004; De Wet et al., 2009; Wakeling et al., 2011; McLean et al., 2017). Most studies suggest that the overall prevalence of feline hyperthyroidism is 2-4%, and at least 6% when only cats older than 9 years of age are considered, but much higher rates have been reported, such as 20% in Poland (Gójska Zygnier et al., 2014). The prevalence of hyperthyroidism in the United Kingdom was noted as 11.9% in a 2005 study of cats >9 years of age and 6% in a 2011 study (Wakeling and Melian, 2005; Wakeling et al., 2011). Two larger subsequent studies in cats from the same country conducted in 2014, reported a prevalence of 3% among 3,584 cats non classified by age and 2.4% or 8.7% in cats older than 10 years of age (O'Neill et al., 2014; Stephens et al., 2014). In North America, the prevalence of feline hyperthyroidism has been reported to increase from 0.1% in 1978-1982 to 2% in 1993-1997 (Edinboro et al., 2004). In New York, the prevalence was 0.3% in 1979 and 4.5% in 1985 (Scarlett et al., 1988). Prevalence studies on feline hyperthyroidism in Germany have also substantiated that the incidence is increasing. In detail, a prevalence of 0.2% was reported in 1987-1994, of 2.6% in 1998, of 11.4% in 2006 in cats older than 8 years of age and of 12.3% in 2016 (Kraft and Buchler, 1999; Sassnau, 2006; Köhler et al., 2016).

Additional epidemiological studies have estimated the prevalence in other countries and suggest a wide geographical variation. In a 2002 study in cats from Japan older than 9 years of age, the prevalence was 8.9% (Miyamoto et al., 2002), and in a 2009 study of cats older than 10 years of age from the same country, it was 3.9% (De Wet et al., 2009). In a 2005 publication from Spain, a prevalence of 1.5% was reported in cats older than 9 years, whilst in Portugal it was 9% among cats with the same age range (Wakeling and Melian, 2005; Dias Neves and Horspool, 2014). In a study from Poland, a prevalence of 20.1% was found among cats older than 10 years of age (Gójska Zygnier et al., 2014) and, more recently, a prevalence of 21.1% in Irish cats aged 10 years or older was reported (Bree et al., 2018).

The variability of prevalence among these studies may reflect differences in the demographics and the geographic origin of the cats, in the accuracy of the diagnostic tests, and thyroid hormone concentration cut-offs employed for diagnosing hyperthyroidism and/or the overtime changes in the prevalence of the disease.

### *Risk factors*

In several studies, old age and indoor lifestyle have been reported as risk factors for feline hyperthyroidism (Martin et al., 2000; Edinboro et al., 2004; Olczak et al., 2005). These findings may reflect chronic exposure to goitrogens, which, in turn, increases the risk of genetic mutations in the thyrocytes, resulting in adenomas or adenomatous hyperplasia. Two epidemiological studies have shown that Siamese cats have significantly lower risk of developing hyperthyroidism compared to other breeds (Scarlett et al., 1988; Kass et al., 1999) and three additional studies have demonstrated a decreased risk in purebred cats (Olczak et al., 2005; De Wet et al., 2009; Wakeling et al., 2009). Regarding gender predisposition, conflicting data exist. Most studies found no sex predilection (Scarlett et al., 1988; Broussard et al., 1995; Kass et al., 1999), but in two studies a female predisposition (Edinboro et al., 2004; Olczak et al., 2005), and in one study a male predisposition (Sassnau, 2006) was reported.

Consumption of commercial canned food has been reported as a risk factor (Scarlett et al., 1988; Broussard et al., 1995; Edinboro et al., 2004; Wakeling et al., 2007; Peterson and Broome, 2015; van Hoek et al., 2015) and has been attributed to the plasticizer linings of the cans that can release the thyroid disruptor chemical bisphenol A into the food during the heating process (Hammarling et al., 2000; Kang and Kondo, 2002; Goodson et al., 2004). In addition, regular use of ectoparasiticides applied directly to the cat or to the cat's environment has been identified as risk factor for feline hyperthyroidism (Scarlett et al., 1988; Peter et al., 1991; Kass et al., 1999; Peterson and Ward, 2007). Finally, use of cat litter has also been reported as a risk factor, but a direct cause-effect relationship has not been established (Hoenig et al., 1982; Kass et al., 1999).

### **7.2. Clinical signs and clinicopathologic abnormalities**

A wide range of manifestations is associated with feline hyperthyroidism, reflecting the generalized and multi-systemic nature of the disease. Weight loss is the most common clinical sign, being present in up to 88% of the cases. Weight loss often appears despite increased appetite, which is reported in up to 49% of hyperthyroid cats. In addition polyuria/polydipsia are also common signs being present in up to 36% of the cases (Broussard et al., 1995). Gastrointestinal manifestations of feline

hyperthyroidism include intermittent vomiting (up to 44% of the cases), diarrhea and increased volume and frequency of defecations and steatorrhea (Peterson and Ferguson, 1983; Thoday and Mooney, 1992). Behavioral changes are seen in up to 50% of cases: hyperthyroid cats may appear hyperactive, aggressive, or exhibit signs of anxiety. In addition, panting, impaired stress tolerance, heat intolerance and dermatologic problems have also been reported.

Palpable goiter is present in most cats with hyperthyroidism. In over 90% of cases of feline hyperthyroidism, either unilateral or bilateral thyroid enlargement is present (Peterson, 2013) since all hyperthyroid cats have either adenomas or carcinomas as the underlying cause of the disease.

Less commonly apathetic hyperthyroidism is diagnosed, and it is characterized by lethargy, weakness, and decreased appetite (Thoday and Mooney, 1992; Bucknell, 2000; Peterson, 2013).

The most well-documented hematological and serum biochemistry abnormalities associated with feline hyperthyroidism include mild anemia, increased alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities, and sometimes mild to moderate increases in blood urea nitrogen (BUN) and creatinine concentrations (Peterson and Ferguson, 1983; Thoday and Mooney, 1992; Broussard et al., 1995).

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## PART TWO-OUR STUDY

### 1. Aims of the study

In Greece, there are few or no reports on the prevalence, the risk factors, and the clinical and clinicopathological manifestations of infections with FeLV, FIV, FCoV, *Bartonella* spp., *Mycoplasma* spp. and *T. gondii*, whereas the prevalence of feline hyperthyroidism has not been reported. For this reason, we conducted a prospective, cross-sectional study, in different populations of cats living in various areas of the country, to: a) determine the seroprevalence for FeLV, FIV, FCoV, *B. henselae* and *T. gondii* and the molecular prevalence of *Bartonella* spp. and *Mycoplasma* spp. infections; b) determine the prevalence of feline hyperthyroidism; c) investigate risk factors for FeLV, FIV, FCoV, *B. henselae* and *T. gondii* seropositivity; d) investigate risk factors for *Bartonella* spp. and *Mycoplasma* spp. PCR-positivity; e) determine the clinical signs, hematological and biochemical findings associated with FeLV, FIV, FCoV, *B. henselae* and *T. gondii* seropositivity; f) determine the clinical signs, hematological and biochemical findings associated with PCR-positivity for *Bartonella* spp. and *Mycoplasma* spp.; g) investigate possible associations between seropositivity for *B. henselae*, PCR-positivity for *Bartonella* spp. or PCR-positivity for *Mycoplasma* spp. and seropositivity for other infectious agents, including *T. gondii*, FCoV, FeLV and FIV; h) to compare the clinical signs, hematological and serum biochemical findings between hyperthyroid and non-hyperthyroid cats.

## **2. Article No 1**

K.G. Kokkinaki, M.N. Saridomichelakis, L. Leontides, M.E. Mylonakis, A. O. Konstantinidis, J.M. Steiner, J.S. Suchodolski, P.G. Xenoulis

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## A prospective epidemiological, clinical, and clinicopathologic study of feline leukemia virus and feline immunodeficiency virus infection in 435 cats from Greece

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### ABSTRACT

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are retroviruses causing significant morbidity and mortality in cats. The aim of this study was to describe the epidemiological, clinical and clinicopathologic aspects of FeLV and FIV infections in different populations of cats in Greece, including client-owned cats, stray cats and cats who live in catteries.

A total of 435 cats were prospectively enrolled. Serological detection of FeLV antigen and FIV antibody was performed using a commercial in-house ELISA test kit.

The results showed that 17 (3.9 %) and 40 (9.2 %) of the 435 cats were positive for FeLV antigen and FIV antibody, respectively, whereas 5 (1.1 %) had concurrent infection with FeLV and FIV. Factors that were associated with FeLV antigenemia, based on multivariate analysis, included vomiting, rhinitis, infection with FIV, neutropenia, decreased blood urea nitrogen and increased serum cholesterol and triglyceride concentrations. Factors associated with FIV seropositivity included male gender, older age, outdoor access, weight loss, fever, gingivostomatitis, skin lesions and/or pruritus and hyperglobulinemia.

Various clinical signs and laboratory abnormalities were found to be significantly associated with retroviral infections, suggesting that current guidelines to test all sick cats should be followed, taking into particular consideration the high-risk groups of cats found in this study.

### 1. Introduction

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are retroviruses, causing significant morbidity and mortality in cats. A wide range of disorders are associated with FeLV or FIV infections, including lymphoma, blood dyscrasias, neurological dysfunction, immune-mediated diseases, immunosuppression with secondary opportunistic infections, gingivostomatitis, reproductive disorders and fading kitten syndrome [1–4]. Reported risk factors for FeLV antigenemia and FIV seropositivity include male gender, outdoor access and unhealthy status [5–8]. The most well-documented clinicopathologic abnormalities associated with retroviral infections in cats include

anaemia, leukopenia, neutropenia and lymphopenia, reduced serum albumin/globulin ratio (A/G ratio), and hypergammaglobulinemia [9].

Epidemiological data are necessary to design optimal strategies for the control of these viral infections. Based on available data, organizations such as the European Advisory Board on Cat Diseases (ABCD) [10] and the American Association of Feline Practitioners (AAFP) [11] have published guidelines for the prevention and management of FeLV and FIV. The prevalence of FeLV antigenemia and FIV seropositivity varies considerably, ranging from 0% to 22.3 % for FeLV and from 0% to 23 % for FIV, depending on the geographical location and the study population [5,12–21].

Only limited seroepidemiological data are available on the retroviral

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infections of cats in Greece, including three studies [22–24] and a preliminary report [25]. Risk factors for FeLV antigenemia and FIV seropositivity and associated clinicopathologic abnormalities were not thoroughly investigated in any of these studies. In addition, they included cats living in certain areas of the country and admitted to veterinary clinics, likely representing the general feline population suboptimally.

The objectives of the present study were: 1) to determine the prevalence of FeLV and FIV infections in different populations of cats living in different parts of Greece; 2) to assess signalment risk factors for FeLV antigenemia and FIV seropositivity; 3) to determine the clinical signs and haematological and biochemical findings associated with FeLV antigenemia and/or FIV seropositivity in cats.

## 2. Materials and methods

### 2.1. Ethics approval

The study protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Thessaly (13/16-6-15). Handling of these animals was in compliance with the European Communities Council Directive 2010/63/EU and state laws.

### 2.2. Study population

Cats from four different geographic areas of Greece (i.e., Attica, Thessaly, Crete, and Macedonia) were prospectively enrolled by three clinicians between November 2013 and November 2016 and divided into three groups (i.e., client-owned, stray, and cats living in catteries). Client-owned cats and cats living in catteries were presented by their owners for wellness examination, vaccination, neutering and/or medical treatment. Two volunteer cat-rescue groups provided stray cats from urban and rural areas that were brought in for clinical examination, vaccination, neutering, and/or treatment. The stray cats often originated from cat-dense environments (i.e., cat colonies) and their age was estimated based on body size, dentition and other physical characteristics [26–28].

The sampling size was calculated using the Epi Info 7 software (Centers of Disease Control and Prevention, Atlanta, Georgia) with a confidence interval of 95 %, expected prevalence of 50 % and maximum error acceptable of 5 %. The minimum number of systematically sampled cats was 384 cats.

Inclusion criteria included: a) body weight of  $\geq 0.5$  kg; and b) an informed consent form signed by the owner or the rescuer. The cats were enrolled randomly and no preference was given to their health status. Signalment and historical data were collected using a standardized questionnaire and a thorough physical examination was performed for each cat by one of three authors (KGK, PGX, AOK).

### 2.3. Sample collection and laboratory analyses

A total of 5 mL of blood was collected by jugular venipuncture from each cat. One milliliter of blood was immediately transferred into EDTA-anticoagulated tube and was used for haematology, whereas serum was separated and stored at  $-80^{\circ}\text{C}$  until biochemical analysis. Haematology was performed utilizing one of three different hematology analyzers (Sysmex poch-100i, ADVIA 2120i Siemens, ADVIA 120 Siemens), depending on the geographic area of sampling and the results were classified as normal, increased, or decreased, based on the reference intervals for each laboratory. For most cats, differential cell count was calculated manually utilizing Diff-Quik (Merc, Germany) stained blood smears, whereas for the remaining cats it was based on the results of analysis using the ADVIA 2120i analyzer. All hematological analyses were performed within 12 h from blood collection. Biochemical analyses were performed using an automated chemistry analyzer (Roche/Hitachi MODULAR ANALYTICS D 2400 module, Roche Diagnostics,

Switzerland, CH).

FeLV antigen and FIV antibody testing were performed using an in-house ELISA assay (SNAP FIV/FeLV Combo test, IDEXX Laboratories, Westbrook, ME, USA) at the time of blood collection, using serum or plasma. The sensitivity and specificity reported by the manufacturer is 98.6 % and 98.2 %, respectively, for FeLV, and 93.5 % and 100 %, respectively, for FIV [29].

### 2.4. Statistical analysis

In the univariate analysis, categorical data regarding signalment, historical information, clinical signs, hematology and biochemistry of FeLV- or FIV- positive cats were compared with those of FeLV or FIV negative cats by either Pearson's  $\chi^2$  or Fisher's exact test. The normality of the distribution of the continuous variables was tested using the Kolmogorov-Smirnov test. Normally distributed data are presented as means  $\pm$  standard deviation and were compared between infected and non-infected cats using independent sample t-tests. Non-normally distributed data are presented as medians and ranges and were compared between infected and non-infected cats using the Mann Whitney U tests.

Variables that were different, at 25 % level of significance, between FeLV- or FIV-positive and negative cats in the univariate analysis were selected as initial candidates for four separate logistic regression models using stepwise, forward and backward selection procedures. Variables identified as being associated with infection status of the cat (at  $P < 0.05$ ) were retained in the final models. Odds ratios (OR) derived from the models were interpreted as measures of increased risk of infection.

The analyses were done using Stata 13 (Stata Corp, College Station, TX) and SPSS 23 for Windows (IBM Corp, Armonk, NY).

## 3. Results

A total of 435 cats were included in the study. The age of these cats ranged from 6 weeks to 17 years (median 2 years). Two hundred and twenty-nine cats (52.6 %) were male (91 neutered) and 206 (47.4 %) were female (65 neutered). Twenty-one cats (4.8 %) were purebred and 399 (91.7 %) were crossbreed, while for 15 (3.5 %) cats these data were missing. One hundred and twenty-one cats (27.8 %) lived exclusively indoors and 292 (67.1 %) outdoors, while for 22 cats (5.1 %) these data were missing. Three hundred and four cats (69.9 %) were living in Attica, 58 (13.3 %) in Thessaly, 45 (10.3 %) in Crete, and 28 (6.4 %) in Macedonia. Two hundred and eighty-six cats (65.7 %) were client-owned, 123 (28.3 %) were stray and 15 (3.5 %) were living in catteries, while for 11 cats (2.5 %) these data were missing. Three hundred and seventy-three cats (85.7 %) lived in urban areas and 37 (8.5 %) in rural areas, while for 25 (5.8 %) cats these data were missing. One hundred and fifty-seven cats (36.1 %) were clinically healthy and 268 (61.6 %) were sick, while for 10 cats (2.3 %) these data were missing.

Of the 435 cats, a total of 17 (3.9 %) and 40 (9.2 %) were found to be positive for FeLV antigen and FIV antibody, respectively. Of these infected cats, 5 (1.1 % of the total 435 cats) had concurrent infections with FeLV and FIV. The prevalence for FeLV and FIV infection was 3.9 % (12/304; 95%CI 3.8 %–4.1 %) and 9.9 % (30/304; 95%CI 9.7 %–10 %) for cats living in Attica, 3.4 % (2/58; 95%CI 2.8 %–4 %) and 12.1 % (7/58; 95%CI 11 %–13.2 %) for cats living in Thessaly, 4.4 % (2/45; 95%CI 3.5 %–5.3 %) and 2.2 % (1/45; 95%CI 1.6 %–2.8 %) for cats living in Crete and 3.6 % (1/28; 95%CI 2.3 %–4.9 %) and 7.1 % (2/28; 95%CI 5.3 %–8.9 %) for cats living in Macedonia, respectively. Based on univariate analyses there was no statistically significant difference in the prevalence of FeLV or FIV infection among the four sampling regions. The prevalence for sick cats was 6% (95%CI 3.1 %–8.8 %) for FeLV infection, 11.2 % (95%CI 7.4 %–15 %) for FIV and 1.5 % (95%CI 0.05 %–3 %) for both viruses. For clinically healthy cats the prevalence was 0% for FeLV and 5.7 % (95%CI 2.1 %–9.3 %) for FIV.

The univariate associations between FeLV antigenemia (Tables S1,



S3, S5, S7) or FIV seropositivity (Tables S2, S4, S6, S8) and signalment-historical data, clinical signs, results of haematology and results of serum biochemistry are presented in supplementary files.

Several factors were found to be significantly associated with FeLV antigenemia in the multivariate analysis (Table 1). Cats with vomiting (OR, 9.25;  $P = 0.003$ ) or rhinitis (OR, 7.33;  $P = 0.002$ ) were significantly more likely to be FeLV-positive compared to cats without vomiting or rhinitis. In addition, infection with FIV increased the risk of infection with FeLV (OR, 3.76;  $P = 0.04$ ). Neutropenic cats (OR, 8.99;  $P = 0.005$ ) and cats with decreased blood urea nitrogen (BUN) concentration (OR, 6.24;  $P = 0.009$ ) were significantly more likely to be FeLV-positive compared to cats with neutrophil counts and BUN concentration within the reference intervals. Finally, cats with increased serum cholesterol (OR, 15.82;  $P = 0.03$ ) or increased serum triglyceride concentration (OR, 9.17;  $P = 0.004$ ) were significantly more likely to be FeLV-positive compared to cats with cholesterol and triglyceride concentrations within the reference intervals.

Multivariate analysis also indicated several factors associated with FIV-seropositivity (Table 2). FIV-positive cats (median age 4.5 years; range 0.13–14 years) were significantly older (OR, 1.17;  $P < 0.001$ ) than FIV-negative cats (median age 2 years; range 0.13–17 years). In addition, male cats (OR, 4.00;  $P = 0.002$ ) and cats living outdoors (OR, 2.67;  $P = 0.04$ ) were significantly more likely to be seropositive than female cats and cats living indoors, respectively. Weight loss (OR, 2.98;  $P = 0.013$ ) and the presence of fever (OR, 5.31;  $P = 0.025$ ), gingivostomatitis (OR, 3.61;  $P = 0.005$ ) and skin lesions and/or pruritus (OR, 2.21;  $P = 0.05$ ) were also significantly associated with FIV-seropositivity. Also, cats with hyperglobulinemia were more likely to be FIV-seropositive (OR, 5.04;  $P < 0.001$ ) compared to cats with normal or reduced serum globulin concentrations.

#### 4. Discussion

This is one of the largest prospective studies on FeLV and FIV infections in cats. The prevalence of these infections varies greatly among countries and regions and depends on the population tested. Studies have reported an overall prevalence of FeLV infection of 22.3 % in Latin America [20], 2.3%–3.3% in North America [19,30], 0%–2.9% in Asia [14,15], and 0 %–15.6 % in Europe. A prospective European study on the prevalence of FeLV infection conducted in 32 countries showed an overall prevalence of 2.3 % [21]. The prevalence of FIV infection has been reported to be 5.8 % in Latin America [20], 2.5 %–23 % in North America [17,19], 0 %–22 % in Asia [13,14], and 2.3 %–11.3 % in Europe [12,18].

**Table 1**

Multivariate analysis of historical data, clinical signs, and results of hematology and serum biochemistry, identified as factors associated with feline leukemia virus (FeLV) antigenemia.

Variables	Categories	OR	CI	P value
Vomiting	No	Reference		
	Yes	9.25	2.11 – 40.54	0.003
Rhinitis	No	Reference		
	Yes	7.33	2.12 – 25.32	0.002
FIV infection	No	Reference		
	Yes	3.76	1.03 – 13.67	0.04
Neutrophils	Decreased	Reference		
	Normal	0.11	0.02 – 0.52	0.005
	Increased	0.28	0.04 – 1.65	0.16
BUN	Decreased	Reference		
	Normal	0.16	0.04 – 0.63	0.009
	Increased	0.67	0.12 – 3.73	0.65
Cholesterol	Normal or decreased	Reference		
	Increased	15.82	1.27 – 196.16	0.03
Triglycerides	Normal or decreased	Reference		
	Increased	9.17	2.03 – 41.24	0.004

OR: odds ratio; CI: confidence interval; FIV: feline immunodeficiency virus; BUN: blood urea nitrogen.

**Table 2**

Multivariate analysis of signalment, historical data, clinical signs, and the results of hematology and serum biochemistry identified as factors associated with feline immunodeficiency virus (FIV) seropositivity.

Variables	Categories	OR	CI	P value
Sex	Female	Reference		
	Male	4	1.69 – 9.36	0.002
Age (years)		1.17	1.07 – 1.28	< 0.001
Living conditions	Indoors	Reference		
	Outdoors	2.67	1.03 – 6.88	0.04
Weight loss	No	Reference		
	Yes	2.98	1.25 – 7.1	0.013
Fever	No	Reference		
	Yes	5.31	1.23 – 22.85	0.025
Gingivostomatitis	No	Reference		
	Yes	3.61	1.47 – 8.88	0.005
Skin lesions and/or pruritus	No	Reference		
	Yes	2.21	0.96 – 5.06	0.05
Globulins	Normal or decreased	Reference		
	Increased	5.04	2.37 – 10.69	< 0.001

OR: odds ratio; CI: confidence interval.

We found an overall prevalence of 3.9 % (95%CI 2.1 %–5.7 %) and 9.2 % (95%CI 6.4 %–11.9 %) for infection with FeLV and FIV, respectively. These percentages are practically the same with those reported in a study from Greece, that assessed the *Leishmania infantum* infection status in 50 clinically healthy cats and 50 sick cats. The authors reported a prevalence of 3% and 8% for FeLV and FIV infection, respectively [24]. In the current study the prevalence for sick cats was 6% for FeLV and 11.2 % for FIV infection. These percentages are quite the same with those reported in a Congress presentation of the results of a retrospective study of 422 sick cats admitted to a veterinary teaching hospital [25]. The authors of the above mentioned presentation reported a prevalence of 4.5 % for FeLV and 10.2 % for FIV.

Our study showed that cats with a history of vomiting were 9.25 times more likely to be FeLV-positive than cats with no such history. To our knowledge, there is no published information suggesting a significant association between vomiting and FeLV infection and the design of our study cannot confirm a causal association. Feline leukemia virus is a potential cause of enteritis, singly or in combination with other enteric pathogens, such as feline panleukopenia virus and coronavirus [31–33].

FeLV-infected cats often have concurrent bacterial, viral, protozoal, and/or fungal infections [31,34,35], including feline upper respiratory track disease (FURTD). In our study, rhinitis was found to be associated with FeLV antigenemia (OR, 7.33) and this is consistent with the results of a retrospective study of 62,000 cats from the United States and Canada, where FeLV-positive cats were 5 times more likely to have respiratory signs compared to non-infected cats [8]. In addition, in two other studies, FURTD affected 18 % or 11 % of FeLV-infected cats, respectively [36,37], but no statistically significant association was found. A more recent European study reported a prevalence of 15 % for FURTD among FeLV-positive cats [21], again without showing a significant association.

Regression analysis showed that FIV infection was a significant risk factor for FeLV infection, and this is consistent with the results of previous studies [5,38]. This finding could be explained by the fact that FIV can cause immunosuppression, therefore increasing the risk for FeLV infection [39].

FeLV infection is associated with a variety of clinicopathologic abnormalities. An important finding was that cats with neutropenia were 9 times more likely to be FeLV-positive compared to cats with normal neutrophil counts. Neutropenia is a common hematological abnormality in FeLV-infected cats and several studies have showed similar results [9, 40]. Neutropenia can be caused by immune-mediated mechanisms, bone



marrow aplasia, myelodysplasia, myelofibrosis or myelophthisis. In FeLV-infected cats, myeloid hypoplasia of all granulocytic stages and an arrest in bone marrow maturation at the myelocyte and metamyelocyte stages can occur [41,42]. In addition, neutrophil counts of some neutropenic FeLV-infected cats recover with glucocorticoid treatment (glucocorticoid-responsive neutropenia), suggesting an immune-mediated pathogenetic mechanism [43].

The reason for the increased prevalence of low serum BUN concentration in FeLV-infected cats is not clear. Theoretically, chronic consumption of low-protein diets or protein malabsorption can lead to low BUN concentrations. This might be especially applicable for stray cats. Interestingly, univariate analysis showed that anorexia was associated with increased risk of FeLV-antigenemia (Supplementary Table S3). Because prolonged anorexia can also lead to reduced protein intake, low BUN could reflect the anorexia associated with FeLV infection or other comorbidities.

The prevalence of increased cholesterol and triglyceride concentrations was significantly higher in FeLV-positive than in FeLV-negative cats. Fasting was not an inclusion criterion for our study and therefore, these results need to be interpreted with caution. Hyperlipidaemia has been associated with several inflammatory conditions in both humans and dogs (e.g., pancreatitis, parvoviral enteritis) and inflammation due to FeLV could potentially have led to increased serum lipid concentrations [44–46].

Male cats were approximately 4 times more likely to be FIV-positive than female cats. Male sex has also been described as an important risk factor for FIV infection in other studies [5,8,19,47,48]. From a behavioral perspective, male cats have been shown to be more aggressive than female cats, leading to a greater risk of bite wounds and FIV transmission.

In the current study FIV positive cats were significantly older than FIV negative cats and this is consistent with previous studies [19,38,49,50]. Older cats are more commonly infected with FIV possibly due to the fact that compared to FeLV, FIV has a longer incubation period, within which cats could remain in the asymptomatic phase for years with a low impact on morbidity and mortality rates.

Outdoor access was also found to be a risk factor for FIV-seropositivity and this is consistent with results of several previous reports [5,8,19,51,52]. Cats living outdoors or having outdoor access have an increased chance to interact with infected cats.

Weight loss was significantly associated to FIV-seropositivity and this finding is in accordance with the results of previous studies [47,53–55]. Weight loss leading to a poor body condition score may be the result of anorexia associated with the infection or comorbidities that developed as a result of the FIV (e.g., neoplasia, secondary infections).

Fever was also found to be significantly associated with FIV-seropositivity. To our knowledge, this is the first time a significant association between the presence of fever and natural FIV infection has been reported. In the first weeks to months after experimental infection, fever, lasting for a few days to a few weeks, may be observed but it is likely to go unobserved by the owners of many naturally infected cats [47]. However, since fever is a non-specific finding that can be present in several diseases in cats [56] this finding need to be interpreted with caution. Fever could be associated with FIV infection as we mentioned previously, but also could be associated with other comorbidities.

Gingivostomatitis is one of the most typical clinical findings in FIV-infected cats and has been attributed to an immune response to chronic antigenic stimulation or to immune dysregulation [2]. Our study indicates a strong association between FIV-seropositivity and gingivostomatitis (OR 3.6), and this is in agreement with previous reports [53,57–59,55]. Furthermore, among client-owned cats, the prevalence of FIV-seropositivity was 5.7 times higher in the cats with oral disease, including stomatitis and gingivitis [60].

Another finding of our study was the higher prevalence of FIV infection among cats with skin lesions and/or pruritus. This is not surprising because many bacterial, viral, and/or fungal infections have

been associated with FIV [52,61]. Bacterial skin diseases that have been associated with FIV infection include folliculitis, superficial spreading pyoderma, abscesses, paronychia and otitis. Fungal, viral, and parasitic infections, such as dermatophytosis, dermatophyte pseudomycetoma and infections with *Candida*, *Malassezia*, *Demodex cati* and cow-poxvirus, have also been associated with FIV infection. In addition, FIV-infected cats may be at risk for developing immune-mediated vasculitis and auricular chondritis. Idiopathic lymphocytic mural folliculitis, plasma cell pododermatitis, and generalized papulocrustous eruption with alopecia and scaling have been reported in FIV-positive cats. Tumors, such as B-cell lymphomas, squamous cell carcinoma, and mast cell tumors also have been reported in association with FIV infection [8,60,62–64].

Similar to others studies, we found that FIV-infected cats had a significantly increased risk of hyperglobulinemia [9,41,65,66]. This finding is a direct consequence of FIV infection, reflects polyclonal B cell activation and has been documented in both natural and experimental infections [67].

## 5. Conclusions

This study demonstrates a relatively high prevalence of FeLV antigenemia and FIV seropositivity of cats in Greece. Thus, it is necessary to establish optimal strategies for prevention and management of infection with these viruses, taking into particular consideration the high-risk groups of cats identified in this study. FeLV infection was associated with vomiting, rhinitis, FIV infection, neutropenia, low serum BUN concentration, hypercholesterolemia, and hypertriglyceridemia. Male gender, older age, and outdoor access were risk factors for FIV-seropositivity. Weight loss, fever, gingivostomatitis, skin lesion and/or pruritus and hyperglobulinemia were associated with FIV-seropositivity. These results can be used to focus testing resources on cats at high risk of infection, when it is not feasible to test all cats as recommended.

## Declaration of Competing Interest

IDEXX Laboratories and Petline supplied the SNAP FIV/FeLV Combo tests used in this study. IDXX Laboratories and Petline did not participate in the study design, sample collection and analysis, or interpretation of data, and in the decision to submit the manuscript for publication. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.cimid.2021.101687>.

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**Supplementary data-Table S1.** Univariable associations between the infection status of 435 cats tested for feline leukemia virus (FeLV) and signalment and historical data.

Variables	Categories	Missing data	FeLV infection status		P value
			Infected (%)	Non-infected (%)	
Sex	Male	0	10/17 (58.8%)	219/418 (52.4%)	0.603
	Female		7/17 (41.2%)	199/418 (47.6%)	
Neutered		8	7/15 (46.7%)	150/412 (36.4%)	0.418
Breed	Purebred	15	0/16 (0%)	21/404 (5.2%)	1
	Crossbreed		16/16 (100%)	383/404 (94.8%)	
Age (years)		29	3 (0.33-12)	2 (0.1-17)	0.119
Body weight (kg)		45	2.5 (1.5-5.7)	3.3 (0.5-9)	0.123
Cat acquisition	Client-owned	73	3/16 (18.8%)	52/346 (15%)	0.809
	Stray		13/16 (81.3%)	272/346 (78.6%)	
	Cattery		0/16 (0%)	19/346 (5.5%)	
	Pet shop		0/16 (0%)	3/346 (0.9%)	
Current ownership	Client-owned	11	12/17 (70.6%)	274/407 (67.3%)	1
	Stray		5/17 (29.4%)	118/407 (29%)	
	Cattery		0/17 (0%)	15/407 (3.7%)	
Living conditions	Indoors	22	2/16 (12.5%)	118/397 (29.7%)	0.168
	Outdoors		14/16 (87.5%)	279/397 (70.3%)	
Living area	Urban	25	13/16 (81.3%)	359/394 (91.1%)	0.177
	Rural		3/16 (18.8%)	35/394 (8.9%)	
Geographic region	Attica	0	12/17 (70.6%)	292/418 (69.9%)	0.967
	Thessaly		2/17 (11.8%)	55/418 (13.2%)	
	Crete		2/17 (11.8%)	43/418 (10.3%)	
	Macedonia		1/17 (5.9%)	28/418 (13.2%)	
Contact with other cats		66	12/14 (85.7%)	284/355 (80%)	1
Number of in-contact cats		118	7 (0-30)	4 (0-46)	0.532
History of cat-fight trauma		243	3/7 (42.9%)	43/185 (23.2%)	0.361

Vaccinated CHPR	against	96	10/14 (71.4%)	185/325 (56.9%)	0.282
Vaccinated FeLV	against	101	0/14 (0%)	14/320 (4.4%)	1

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CHPR: calicivirus, herpesvirus-1, panleukopenia, rabies.

**Supplementary data-Table S2.** Univariable associations between the infection status of 435 cats tested for feline immunodeficiency virus (FIV) and signalment and historical data.

Variables	Categories	Missing data	FIV infection status		<i>P</i> value
			Infected (%)	Non-infected (%)	
Sex	Male	0	31/40 (77.5%)	198/395 (50.1%)	0.001
	Female		9/40 (22.5%)	197/395 (49.9%)	
Neutered		8	22/38 (57.9%)	135/389 (34.7%)	0.005
Breed	Purebred	15	2/37 (5.4%)	19/383 (5%)	0.706
	Crossbreed		35/37 (94.6%)	364/383 (95%)	
Age (years)		29	4.5 (0.13-15)	2 (0.1-17)	<0.001
Body weight (kg)		45	4 (0.58-9)	3.2 (0.5-8.5)	0.026
Cat acquisition	Client-owned	73	2/33 (6.1%)	53/329 (16.1%)	0.118
	Stray		27/33 (81.8%)	258/329 (78.4%)	
	Cattery		4/33 (12.1%)	15/329 (4.6%)	
	Pet shop		0/33 (0%)	3/329 (0.9%)	
Current ownership	Client-owned	11	24/39 (61.5%)	262/385 (68.1%)	0.307
	Stray		12/39 (30.8%)	111/385 (28.8%)	
	Cattery		3/39 (7.7%)	12/385 (3.1)	
Living conditions	Indoors	22	6/36 (16.7%)	114/377 (30.2%)	0.087
	Outdoors		30/36 (83.3%)	263/377 (69.5%)	
Living area	Urban	25	36/36 (100%)	336/374 (89.8%)	0.037
	Rural		0/36 (0%)	38/374 (10.2%)	
Geographic region	Attica	0	30/40 (75%)	274/395 (69.4%)	0.297
	Thessaly		7/40 (17.5%)	50/395 (12.7%)	
	Crete		1/40 (2.5%)	44/395 (11.1%)	
	Macedonia		2/40 (5%)	27/395 (6.8%)	
Contact with other cats		66	24/30 (80%)	272/339 (80.2%)	0.975
Number of in-contact cats		118	4.5 (0-32)	4 (0-46)	0.318
History of cat-fight trauma		243	5/13 (38.5%)	41/179 (22.9%)	0.309

Vaccinated CHPR	against	96	18/29 (62.1%)	177/310 (57.1%)	0.604
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CHPR: calicivirus, herpesvirus-1, panleukopenia, rabies.

**Supplementary data-Table S3.** Univariable associations between the infection status of 435 cats tested for feline leukemia virus (FeLV) and clinical signs.

Clinical sign	Missing data	FeLV infection status		<i>P</i> value
		Infected (%)	Non-infected (%)	
Weight loss	99	4/13 (30.8%)	42/323 (13%)	0.087
Anorexia (previous 3 weeks)	66	7/15 (46.7%)	66/354 (18.6%)	0.015
Depression-lethargy	6	4/16 (25%)	49/413 (11.9%)	0.112
Weakness	6	1/16 (6.3%)	13/413 (3.1%)	0.418
Dehydration	7	5/16 (31.3%)	35/412 (8.5%)	0.011
Fever	12	0/16 (0%)	13/407 (3.2%)	1
Lymphadenomegaly	8	2/16 (12.5%)	24/411 (5.8%)	0.254
Abnormal lung sounds	7	1/16 (6.3%)	9/412 (2.2%)	0.320
Cough	4	0/16 (0%)	6/415 (1.4%)	1
Dyspnea	7	0/16 (0%)	8/412 (1.9%)	1
Signs of rhinitis	7	5/16 (31.3%)	30/412 (7.3%)	0.006
Ocular signs	16	2/15 (13.3%)	41/404 (10.1%)	0.659
Gingivostomatitis	6	3/16 (18.8%)	53/413 (12.8%)	0.451
Glossitis	6	1/16 (6.3%)	14/413 (3.4%)	0.44
Vomiting	88	3/15 (20%)	16/332 (4.8%)	0.042
Diarrhea	93	2/15 (13.3%)	13/327 (4%)	0.136
Constipation	104	0/14 (0%)	3/317 (0.9%)	1
Abdominal pain	6	0/16 (0%)	11/413 (2.7%)	1
Abdominal mass	7	0/16 (0%)	3/412 (0.7%)	1
Abdominal effusion	6	0/16 (0%)	6/413 (1.5%)	1
Hepatomegaly	7	0/16 (0%)	5/412 (1.2%)	1
Splenomegaly	7	0/16 (0%)	2/412 (0.5%)	1
Decreased kidney size	7	0/16 (0%)	2/412 (0.5%)	1
Increased kidney size	7	0/16 (0%)	4/412 (1%)	1
Non-smooth kidney surface	7	1/16 (6.3%)	2/412 (0.5%)	0.108
Polyuria-polydipsia	109	0/14 (0%)	6/312 (1.9%)	1
Dysuria	104	0/14 (0%)	5/317 (1.6%)	1
Pyometra or penile swelling	6	0/16 (0%)	2/413 (0.5%)	1

Skin lesions and/or pruritus	13	6/16 (37.5%)	83/406 (20.4%)	0.118
Neurological signs	7	0/16 (0%)	19/412 (4.6%)	1
Musculoskeletal signs	6	0/16 (0%)	16/413 (3.9%)	1
At least one clinical sign	10	16/16 (100%)	252/409 (61.6%)	0.002
At least one clinical sign compatible with FeLV	10	12/16 (75%)	158/409 (38.6%)	0.004
FIV infection	0	5/17 (29.4%)	35/418 (8.4%)	0.014

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FIV: feline immunodeficiency virus.

**Supplementary data-Table S4.** Univariable associations between the infection status of 435 cats tested for feline immunodeficiency virus (FIV) and clinical signs.

Clinical sign	Missing data	FIV infection status		<i>P</i> value
		Infected (%)	Non-infected (%)	
Weight loss	99	11/30 (36.7%)	35/306 (11.4%)	0.001
Anorexia (previous 3 weeks)	66	15/33 (45.5%)	58/336 (17.3%)	<0.001
Depression-lethargy	6	9/39 (23.1%)	44/390 (11.3%)	0.042
Weakness	6	2/39 (5.1%)	12/390 (3.1%)	0.369
Dehydration	7	8/38 (21.1%)	32/390 (8.2%)	0.017
Fever	12	3/38 (7.9%)	10/385 (2.6%)	0.102
Lymphadenomegaly	8	2/38 (5.3%)	24/389 (6.2%)	1
Abnormal lung sounds	7	0/38 (0%)	10/390 (2.6%)	1
Cough	4	0/39 (0%)	6/392 (1.5%)	1
Dyspnea	7	2/38 (5.3%)	6/390 (1.5%)	0.153
Signs of rhinitis	7	5/39 (12.8%)	30/389 (7.7%)	0.351
Ocular signs	16	6/38 (15.8%)	37/381 (9.7%)	0.258
Gingivostomatitis	6	12/39 (30.8%)	44/390 (11.3%)	0.001
Glossitis	6	3/39 (7.7%)	12/390 (3.1%)	0.147
Vomiting	88	1/33 (3%)	18/314 (5.7%)	1
Diarrhea	93	2/33 (6.1%)	13/309 (4.2%)	0.646
Constipation	104	0/30 (0%)	3/301 (1%)	1
Abdominal pain	6	0/39 (0%)	11/390 (2.8%)	0.609
Abdominal mass	7	0/39 (0%)	3/389 (0.8%)	1
Abdominal effusion	6	1/39 (2.6%)	5/390 (1.3%)	0.438
Hepatomegaly	7	0/39 (0%)	5/389 (1.3%)	1
Splenomegaly	7	0/39 (0%)	2/389 (0.5%)	1
Decreased kidney size	7	0/39 (0%)	2/389 (0.5%)	1
Increased kidney size	7	0/39 (0%)	4/389 (1%)	1
Non-smooth kidney surface	7	1/39 (2.6%)	2/389 (0.5%)	0.25
Polyuria-polydipsia	109	0/30 (0%)	6/296 (2%)	1
Dysuria	104	0/30 (0%)	5/301 (1.7%)	1
Pyometra or penile swelling	6	0/39 (0%)	2/390 (0.5%)	1
Skin lesions and/or pruritus	13	14/39 (35.9%)	75/383 (19.6%)	0.017



Neurological signs	7	1/39 (2.6%)	18/389 (4.6%)	1
Musculoskeletal signs	6	2/39 (5.1%)	14/390 (3.6%)	0.648
At least one clinical sign	10	30/39 (76.9%)	238/386 (61.7%)	0.06
At least one clinical sign compatible with FIV	10	23/39 (59%)	148/386 (38.3%)	0.012
FeLV infection	0	5/40 (12.5%)	12/395 (3%)	0.014

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FeLV: feline leukemia virus.

**Supplementary data-Table S5.** Laboratory values and univariable associations between the infection status of 303 cats tested for feline leukemia virus (FeLV) and haematology.

Variable	Categories	Missing data	FeLV infection status		<i>P</i> value
			Infected (%)	Non-infected (%)	
HCT	Decreased		4/9 (44.4%)	84/294 (28.6%)	0.29
HCT (%; mean; SD)		0	25.96; 8.6	31.86; 7	
WBC	Decreased		3/9 (33.3%)	18/294 (6.1%)	0.018
	Increased		2/9 (22.2%)	76/294 (25.9%)	
WBC (x10 <sup>3</sup> /μL, median; range)		0	12.45; 1.33-20.87	12.24; 33-65.98	
Neutrophils	Decreased		4/9 (44.4%)	31/294 (10.5%)	0.008
	Increased		2/9 (22.2%)	54/294 (18.4%)	
Neutrophils (x10 <sup>3</sup> /μL, median; range)		0	6.85; 1.1-14.47	6.91; 0.46-57.64	
Lymphocytes	Decreased		2/9 (22.2%)	45/294 (15.3%)	0.811
	Increased		0/9 (0%)	20/294 (6.8%)	
Lymphocytes (x10 <sup>3</sup> /μL, median; range)		0	34.85; 0.09-5.12	34.84; 0.92-14.96	
Monocytes	Increased		0/9 (0%)	9/294 (3.1%)	1
Monocytes (x10 <sup>3</sup> /μL, median; range)		0	0.27; 0-0.64	0.268; 0-2.1	
Eosinophils	Decreased		2/9 (22.2%)	10/294 (3.4%)	0.074
	Increased		1/9 (11.1%)	59/294 (20.1%)	
Eosinophils (x10 <sup>3</sup> /μL, median; range)		0	0.98; 0.05-3.51	0.9; 0-75.10	
Platelets	Decreased		3/9 (33.3%)	52/294 (17.7%)	0.433
	Increased		0/9 (0%)	18/294 (6.1%)	
Platelets (x10 <sup>3</sup> /μL, median; range)		0	304; 11-607	303; 18-94.2	

HCT: haematocrit; SD: standard deviation; WBC: white blood cells

**Supplementary data-Table S6.** Laboratory values and univariable associations between the infection status of 303 cats tested for feline immunodeficiency virus (FIV) and haematology

Variable	Categories	Missing data	FIV infection status		<i>P</i> value
			Infected (%)	Non-infected (%)	
HCT	Decreased		10/24 (41.7%)	78/279 (28%)	0.156
HCT (%; mean; SD)		0	29.6; 7	31.87; 7.2	
WBC	Decreased		3/24 (12.5%)	18/279 (6.5%)	0.45
	Increased		7/24 (29.2%)	71/279 (25.4%)	
WBC (x10 <sup>3</sup> /μL, median; range)		0	12.19; 4.6-26.15	12.2; 1.33-65.98	
Neutrophils	Decreased		4/24 (16.7%)	31/279 (11.1%)	0.198
	Increased		7/24 (29.2%)	49/279 (17.6%)	
Neutrophils (x10 <sup>3</sup> /μL, median; range)		0	6.84; 0.46-20.75	6.885; 1.1-57.64	
Lymphocytes	Decreased		6/24 (25%)	41/279 (14.7%)	0.184
	Increased		0/24 (0%)	20/279 (7.2%)	
Lymphocytes (x10 <sup>3</sup> /μL, median; range)		0	3.54; 0.09-10.63	3.487; 0.09-14.96	
Monocytes	Increased		0/24 (0%)	9/279 (3.2%)	1
Monocytes (x10 <sup>3</sup> /μL, median; range)		0	0.268; 0-0.67	0.266; 0-2.1	
Eosinophils	Decreased		2/24 (8.3%)	10/279 (3.6%)	0.344
	Increased		5/24 (20.8%)	55/279 (19.7%)	
Eosinophils (x10 <sup>3</sup> /μL, median; range)		0	0.95; 0-3.92	0.918; 0-7.51	
Platelets	Decreased		5/24 (20.8%)	50/279 (17.9%)	0.73
	Increased		2/24 (8.3%)	16/279 (5.7%)	
Platelets (x10 <sup>3</sup> /μL, median; range)		0	303; 18-812	304; 11-942	

HCT: haematocrit; SD: standard deviation; WBC: white blood cells.

**Supplementary data-Table S7.** Laboratory values and univariable associations between the infection status of 435 cats tested for feline leukemia virus (FeLV) and serum biochemistry.

Variable	Categories	Missing data	FeLV infection status		<i>P</i> value
			Infected (%)	Non-infected (%)	
Total proteins	Decreased		1/13 (7.7%)	28/386 (7.3%)	0.128
	Increased		2/13 (15.4%)	16/386 (4.1%)	
Total proteins	(g/dL, median; range)	36	6.75; 3.94-10.34	6.72; 4.23-11.9	
Albumins	Decreased		12/13 (92.3%)	290/390 (74.4%)	0.199
Albumins	(g/dL, mean; SD)	32	2.71; 0.48	2.91; 0.43	
Globulins	Decreased		1/13 (7.7%)	15/386 (3.9%)	0.113
	Increased		7/13 (53.8%)	122/386 (31.6%)	
Globulins	(g/dL, median; range)	36	3.71; 2.26-8.43	3.71; 2.1-9.82	
Urea nitrogen	Decreased		4/13 (30.8%)	43/383 (11.2%)	0.008
	Increased		3/13 (23.1%)	29/383 (7.6%)	
Urea nitrogen	(mg/dL, median; range)	39	22.61; 13.72-96.74	22.68; 9.22-1300	
Creatinine	Decreased		0/13 (0%)	38/387 (9.8%)	0.338
	Increased		1/13 (7.7%)	13/387 (3.4%)	
Creatinine	(mg/dL, median; range)	35	1.02; 0.6-3.07	1.04; 0.2-10.73	
Glucose	Decreased		1/13 (7.7%)	11/383 (2.9%)	0.344
	Increased		5/13 (38.5%)	214/383 (55.9%)	
Glucose	(mg/dL, median; range)	39	150.28; 59.26-194.17	148.46; 10.11-423.13	
Cholesterol	Decreased		0/13 (0%)	38/381 (10%)	0.105
	Increased		1/13 (7.7%)	3/381 (0.8%)	
Cholesterol	(mg/dL, median; range)	41	112.83; 81.88-259.83	112.4; 42.68-268.98	
Triglycerides	Decreased		0/13 (0%)	7/386 (1.8%)	0.041
	Increased		3/13 (23.1%)	17/386 (4.4%)	

Triglycerides	(mg/dL, median; range)	36	57.08; 35.53- 191.5	56.2; 16.73-231.57	
Total bilirubin	Increased		1/13 (7.7%)	18/389 (4.6%)	0.472
Total bilirubin	(mg/dL, median; range)	33	0.16; 0.01-2.56	0.15; 0-8.49	
ALP	Increased		0/13 (0%)	32/390 (8.2%)	0.611
ALP	(U/L, median; range)	32	26.86; 5.15- 89.41	26.53; 5.29-794.28	
ALT	Increased		1/13 (7.7%)	13/379 (3.4%)	0.381
ALT	(U/L, median; range)	43	17.28; 3.21- 144.75	18.77; 3.05-5000	
AST	Increased		1/13 (7.7%)	41/389 (10.5%)	1
AST	(U/L, median; range)	33	18.08; 9.16- 568.19	19.825; 4.46-577	
GGT	Increased		0/13 (0%)	4/381 (1%)	1
GGT	(U/L, median; range)	41	3; 3	3; 0.35-9.72	
Calcium	Decreased		1/13 (7.7%)	19/388 (4.9%)	
	Increased		0/13 (0%)	4/388 (1%)	0.557
Calcium	(mg/dL, median; range)	34	9.44; 6.7-10.49	9.36; 4.51-40	
Phosphorus	Increased		5/13 (38.5%)	167/382 (43.7%)	0.707
Phosphorus	(mg/dL, median; range)	40	6.33; 4.64-9.47	6.01; 2.79-200	
Potassium	Decreased		0/13 (0%)	7/388 (1.8%)	
	Increased		4/13 (30.8%)	90/388 (23.2)	0.619
Potassium	(mEq/L, median; range)	34	4.81; 4.01-5.92	4.77; 2.53-100	
Sodium	Decreased		3/13 (23.1%)	57/389 (14.7%)	
	Increased		1/13 (7.7%)	39/389 (10%)	0.564
Sodium	(mEq/L, median; range)	33	152.99; 130.19- 168.32	152.75; 134.85-186.16	
Chloride	Decreased		5/13 (38.5%)	127/389 (32.6%)	

	Increased		1/13 (7.7%)	6/389 (1.5%)	0.176
Chloride	(mEq/L, median; range)	33	117.42; 98.08- 135.56	117.41; 102.39-144.53	

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SD: standard deviation; ALP: alkaline phosphatase; ALT: alanine aminotransferase;  
AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase

**Supplementary data-Table S8.** Laboratory values and univariable associations between the infection status of 435 cats tested for feline immunodeficiency virus (FIV) and serum biochemistry

Variable	Categories	Missing data	FIV infection status		<i>P</i> value
			Infected (%)	Non-infected (%)	
Total proteins	Decreased		1/37 (2.7%)	28/362 (7.7%)	0.112
	Increased		4/37 (10.8%)	14/362 (3.9%)	
Total proteins	(g/dL, median; range)	36	6.72; 5.69-10.35	6.72; 3.94-11.9	
Albumins	Decreased		36/38 (94.7%)	266/365 (72.9%)	0.003
Albumins	(g/dL, mean; SD)		2.66; 0.41	2.93; 0.42	
Globulins	Decreased		0/37 (0%)	16/362 (4.4%)	<0.001
	Increased		26/37 (70.3%)	103/362 (28.5%)	
Globulins	(g/dL, median; range)	36	3.71; 2.85-8.43	3.7; 2.1-9.82	
Urea nitrogen	Decreased		5/37 (13.5%)	42/359 (11.7%)	0.282
	Increased		5/37 (13.5%)	27/359 (7.5%)	
Urea nitrogen	(mg/dL, median; range)	39	22.67; 13.48-1300	22.68; 9.22-1300	
Creatinine	Decreased		2/38 (5.3%)	36/362 (9.9%)	0.174
	Increased		3/38 (7.9%)	11/362 (3%)	
Creatinine	(mg/dL, median; range)	35	1.03; 0.25-7.92	1.04; 0.2-10.73	
Glucose	Decreased		1/38 (2.6%)	11/358 (3.1%)	0.754
	Increased		19/38 (50%)	200/358 (55.9%)	
Glucose	(mg/dL, median; range)	39	149.14; 58.66-408.5	148.46; 10.11-423.13	
Cholesterol	Decreased		2/38 (5.3%)	36/356 (10.1%)	0.708
	Increased		0/38 (0%)	4/356 (1.1%)	
		41			
Cholesterol	(mg/dL, median; range)		112.4; 62.49-247.97	112.4; 42.68-268.98	
Triglycerides	Decreased		0/38 (0%)	7/361 (1.9%)	



	Increased		2/38 (5.3%)	18/361 (5%)	1
Triglycerides	(mg/dL, median; range)	36	55.79; 29.45- 162.54	56.32; 16.73-231.57	
Total bilirubin	Increased		2/38 (5.3%)	17/364 (4.7%)	0.698
Total bilirubin	(mg/dL, median; range)	33	0.15; 0-0.46	0.15; 0-8.49	
ALP	Increased		0/38 (0%)	32/365 (8.8%)	0.058
ALP	(U/L, median; range)	32	26.53; 5.15- 85.34	26.53; 5.29-794.28	
ALT	Increased		0/36 (0%)	14/356 (3.9%)	0.628
ALT	(U/L, median; range)	43	18.91; 3.21- 53.91	18.77; 3.05-5000	
AST	Increased		2/38 (5.3%)	40/364 (11%)	0.404
AST	(U/L, median; range)	33	19.76; 4.46- 58.99	19.82; 5.18-577	
GGT	Increased		0/38 (0%)	4/356 (1.1%)	1
GGT	(U/L, median; range)	41	3; 3	3; 0.35-9.72	
Calcium	Decreased		2/38 (5.3%)	18/363 (5%)	
	Increased		1/38 (2.6%)	3/363 (0.8%)	0.405
Calcium	(mg/dL, median; range)	34	9.35; 7.86-40	9.36; 4.51-15.1	
Phosphorus	Increased		7/38 (18.4%)	165/357 (46.2%)	0.001
Phosphorus	(mg/dL, median; range)	40	6.01; 3.06-200	6.01; 2.79-18.77	
Potassium	Decreased		2/38 (5.3%)	5/363 (1.4%)	
	Increased		8/38 (21.1%)	86/363 (23.7%)	0.214
Potassium	(mEq/L, median; range)	34	4.77; 3.06-100	4.775; 2.53-8.95	
Sodium	Decreased		9/38 (23.7%)	51/364 (14%)	
	Increased		3/38 (7.9%)	37/364 (10.2%)	0.274
Sodium	(mEq/L, median; range)	33	152.75; 143.76- 163.99	152.75; 130.19-186.16	

Chloride	Decreased		16/38 (42.1%)	116/364 (31.9%)	
	Increased		0/38 (0%)	7/364 (1.9%)	0.334
Chloride	(mEq/L, median; range)	33	117.38; 109.72- 127.2	117.41; 98.08-144.53	

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SD: standard deviation; ALP: alkaline phosphatase; ALT: alanine aminotransferase;  
AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase

### **3. Article No 2**

**Seroprevalence and risk factors for feline coronavirus infection in cats from Greece. Under review**

## **Short Communication**

### **Seroprevalence and risk factors for feline coronavirus infection in cats from Greece**

## **Abstract**

Feline coronavirus (FCoV) is a highly contagious and ubiquitous virus of domestic cats and wild felids. Feline infectious peritonitis (FIP) is a fatal, systemic disease caused by FCoV when spontaneous mutations of its genome take place.

The aims of this study were to determine the prevalence of seropositivity for FCoV in different populations of cats in Greece and to assess risk factors for seropositivity.

A total of 453 cats were prospectively enrolled and a commercially available IFAT was used for the detection of FCoV IgG in their serum.

Overall, 55 (12.1%) of the 453 cats were seropositive for FCoV. Based on multivariate analysis, risk factors for seropositivity included the cat being adopted as stray and be in contact with other cats.

This is the first extensive study on the epidemiology of FCoV in cats from Greece and one of the largest worldwide. Feline coronavirus seropositivity was found to be common. Therefore, it is necessary to establish optimal strategies for the prevention of FCoV infection, considering the high-risk groups of cats identified in this study.

*Keywords:* cat, infectious peritonitis, prevalence, seropositivity

## Introduction

Feline coronavirus (FCoV) is a highly contagious and ubiquitous virus of domestic cats and wild felids. Feline infectious peritonitis (FIP) is a fatal, systemic disease caused by mutated strains of FCoV.

FCoV strains are subdivided into two biotypes; feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) (Pedersen, 1987). It is now accepted that these biotypes are not different species, but they represent variants of the same virus. Thus, it has been suggested to call the biotypes of FCoV as "the less virulent FCoV" and "the FIP-associated FCoV". The most widely accepted hypothesis of developing FIP is the internal mutation theory. Based on this theory, the less virulent FCoV converts to FIP-associated FCoV in individual cats by genetic modifications that induce, among others, a change in virus tropism from the enterocytes to the macrophages (Barker et al., 2013; Decaro et al., 2021).

FCoV is shed in the feces, and the transmission results from direct ingestion of infected feces or from contaminated litter and other fomites. Reported risk factors for FCoV-seropositivity and FIP include multi-cat environments, young age, being purebred and male (Pedersen, 1976; Rohrbach et al., 2001; Cave et al., 2004; Sharif et al., 2009).

FCoV infection has been reported worldwide and around 40% of domestic cats are infected, although the seroprevalence may be as high as 90% in multi-cat households (Addie and Jarrett, 1992b; Addie, 2000). Furthermore, approximately 5-12% of infected cats will develop FIP (Addie and Jarrett, 1992a; Addie and Jarrett, 1992b; Addie et al., 1995). Therefore, current epidemiological data are necessary to design optimal strategies for the control of the infection. Based on available data, organizations, such as the European Advisory Board on Cat Diseases (ABCD), have published guidelines on the prevention and management of FCoV infection (Addie et al., 2009).

Only limited data are available on FCoV seroprevalence of cats in Greece (Koutinas and Koptopoulos, 1993; Chatzis et al., 2014), and there is no information on the risk factors for seropositivity. Thus, the objectives of the present study are to determine the prevalence of seropositivity for FCoV in different populations of cats living in various regions of Greece, and to investigate the signalment and historical information to identify risk factors for seropositivity.

## **Materials and methods**

### *Ethics approval*

The study protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Thessaly (13/16-6-15). Handling of these animals was in compliance with the European Communities Council Directive 2010/63/EU and state laws.

### *Study population*

Cats from four different geographic areas of Greece (i.e., Attica, Thessaly, Crete and Macedonia) were prospectively enrolled by three clinicians between November 2013 and November 2016, as part of a previous study (Kokkinaki et al., 2021), and they were divided into three groups: client-owned cats, stray cats, and cats living in breeding catteries. Cats were presented by their owners (client-owned or cattery cats) or by two cat-rescue groups (stray cats) for wellness examination, vaccination, neutering and/or medical treatment. Stray cats often originated from cat-dense environments (i.e., rescue colonies), and their age was estimated based on body size, dentition and other physical characteristics (Hale, 2005; Bellows et al., 2016; DiGangi et al., 2020).

Inclusion criteria were: a) body weight of  $\geq 0.5$  kg; and b) an informed consent form signed by the owner or the rescuer. The cats were enrolled randomly, and no preference was given to their health status. Signalment and historical data were collected using a standardized questionnaire.

### *Sample collection and laboratory analyses*

A total of 5 mL of blood was collected by jugular venipuncture from each cat. Serum was harvested following centrifugation at 3000 rpm for 20 min and stored at  $-80^{\circ}\text{C}$  until an indirect fluorescent antibody test (IFAT) was done.

FCoV IgG antibodies were detected using a commercially available IFAT kit (Biopronix Product Line, Agrolabo S.p.a., Italy) and performed according to the manufacturer's instructions. Transmissible gastroenteritis virus (TGEV; a porcine coronavirus) was used in IFAT slides as antigen substrate. The cut-off value was 1/100, and all slides were examined by fluorescence microscopy (Olympus, Japan) at 400x magnification.

### *Statistical analysis*

For univariate analysis, the categorical data from signalment and historical information of FCoV seropositive cats were compared to those of seronegative cats using Pearson's  $\chi^2$  or Fisher's exact tests. The normality of the distribution of the continuous variables was tested using the Kolmogorov-Smirnov test. Normally distributed data are presented as means  $\pm$  standard deviation and were compared between seropositive cats and seronegative cats using independent sample t-tests. Not normally distributed data are presented as medians and ranges and were compared between seropositive cats and seronegative cats using Mann Whitney U tests.

Variables that were different in the univariate analysis, at 25% level of significance, between seropositive and seronegative cats were selected as candidates for an initial logistic regression model. The initial model was subsequently reduced in a stepwise manner until only significant at  $P < 0.05$  variables remained. Odds ratios (OR) derived from the reduced model were interpreted as measures of risk of seropositivity.

The analyses were performed using Stata 13 (Stata Corp, College Station, TX) and SPSS 23 for Windows (IBM Corp, Armonk, NY).

### **Results**

A total of 453 cats were included in the study. The age of these cats ranged from 6 weeks to 17 years (median: two years). Two hundred and forty cats (53%) were male (86 neutered; 35.8% of the male cats), and 213 (47%) were female (52 neutered; 24.4% of the female cats). Fifteen cats (3.3%) were purebred, and 423 (93.4%) were common European cats, while for fifteen (3.3%) cats the breed was not recorded. Ninety-eight cats (21.6%) lived exclusively indoors and 333 (73.5%) outdoors, while for 22 cats (4.9%) living status was not recorded. Two hundred and fifty-four cats (56.1%) were living in Attica, 73 (16.1%) in Thessaly, 79 (17.4%) in Crete, and 47 (10.4%) in Macedonia. Two hundred and sixty-seven cats (58.9%) were client-owned, 156 (34.4%) were stray, and 21 (4.6%) were living in catteries, while for nine cats (2%) the current ownership was not recorded.

Of the 453 cats, a total of 55 (12.1%) were seropositive for FCoV. The univariate associations between seropositivity for FCoV and the signalment or the historical information are presented in Table 1.



Multivariate analysis indicated factors associated with FCoV-seropositivity (Table 2). Cats adopted as strays were significantly more likely (OR, 2.94) to be FCoV-seropositive compared to cats that were adopted as non-stray cats. In addition, cats who had contact with other cats were significantly more likely (OR, 4.54) to be seropositive compared to cats who were not in contact with other cats.

## Discussion

This is one of the most extensive prospective studies on seropositivity against FCoV in cats, and the first study reporting risk factors for seropositivity in Greece. Overall, 12.1% of the cats were seropositive and the risk factors identified included adoption as strays and contact with other cats.

The seroprevalence identified here is considerably higher than the one reported in a previous seroepidemiological study, including 202 cats from Macedonia, Greece, where it was only 5.9% (Koutinas and Koptopoulos, 1993). Even more, in a recent study from Greece, that investigated *Leishmania infantum* infection in 100 cats, reported a 0% prevalence of anti-FCoV seropositivity (Chatzis et al., 2014). Other studies have reported an overall seroprevalence of 0-84% in Europe (Sparkes et al., 1992; Herrewegh et al., 1997), 0-71.6% in America (Pedersen, 1976; Levy et al., 2008), 6.6-28.2% in Asia (An et al., 2011; Wang et al., 2014), 18-40% in Australia (Jones and Hogg, 1974; Watson et al., 1974) and 35% in South Africa (Bland van den Berg and Botha, 1977). The variability of seroprevalence among these studies may reflect differences in the demographics and the geographic origin of the cats, in the accuracy of the diagnostic tests, or the overtime changes in the frequency of exposure of the cats to FCoV.

In our study, cats that were in contact with other cats, either on a permanent basis (constant contact) or occasionally, were significantly more likely to be seropositive, compared to those that were not in contact with other cats. This finding is consistent with the results of several previous reports (Pedersen, 1976; Cave et al., 2004; Bell et al., 2006; Drechsler et al., 2011). In a study from the United Kingdom, authors reported that cats originating from multi-cat households were approximately two times more likely to be FCoV-seropositive compared to cats from single-cat households (Cave et al., 2004). In addition, in a preliminary study on the epidemiology of FCoV in cats from Turkey, the reported seroprevalence rate among cats from multi-cat environments was 62% versus 4% among cats living in single-cat

households (Pratelli et al., 2009). It is well documented that the virus is transmitted via the fecal-oral route; thus, the prevalence of exposure to FCoV increases in parallel with the number and density of in-contact cats (Drechsler et al., 2011).

Cats that had been adopted as strays were significantly more likely to be seropositive compared to cats that were adopted as non-stray cats (i.e., from private owners or from catteries). This finding is in agreement with the results of a study in the United Kingdom, where free-roaming cats were more likely to be FCoV-seropositive compared to indoor cats (Cave et al., 2004). Similarly, in an earlier study from the same country, feral or semi-feral cats were almost twice as likely to be seropositive compared to household cats (Muirden, 2002). On the contrary, many other studies conclude that outdoor access reduces the risk of exposure to FCoV, possibly because outdoor cats have a larger territory and they bury their feces in the soil, thus minimizing fecal-oral transmission of FCoV. However, in urban environments with high cat population densities, communal use of soiling areas may occur, and stray cat movement through several territories may lead to a higher risk of exposure to FCoV.

## Conclusions

Feline coronavirus infection is relatively common in Greece. Cats adopted as strays and those in contact with other cats are at increased risk of seropositivity. These risk factors should be taken into consideration in order to establish optimal strategies for the prevention of FCoV infection.

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**Table 1.** Univariable associations between seropositivity of 453 cats against feline coronavirus (FCoV), and the signalment and historical information that were collected using a standardized questionnaire. Variables that were used in the logistic regression model ( $P$  value  $<0.25$ ) are highlighted with an asterisk

Variables	Categories	Missing data	Serology for FCoV		$P$ value
			Positive (%)	Negative (%)	
Sex	Male	0	30/55 (54.5%)	210/398 (52.8%)	0.804
	Female		25/55 (45.5%)	188/398 (47.2%)	
Neutered		6	18/55 (32.7%)	120/392 (30.6%)	0.751
Breed	Purebred	15	2/54 (3.7%)	13/384 (3.4%)	1
	Crossbreed		52/54 (96.3%)	371/384 (96.6%)	
Age (years)		23	1.75 (0.38-12)	2 (0.13-17)	0.597
Body weight (kg)		45	3.3 (1-7.2)	3.2 (0.5-9)	0.559
Cat acquisition	Stray	93	39/45 (86.7%)	219/315 (69.5%)	0.017*
	Non-stray <sup>†</sup>		6/45 (13.3%)	96/315 (30.5%)	
Current ownership	Client-owned		26/53 (49.1)	241/391 (61.6%)	
	Stray	9	23/53 (43.4%)	133/391 (34%)	0.139*
	Cattery		4/53 (7.5%)	17/391 (4.3%)	
Living conditions	Indoors	22	10/52 (19.2%)	88/379 (23.2%)	0.52
	Outdoors		42/52 (80.8%)	291/379 (76.8%)	
Contact with other cats		58	44/46 (95.7%)	295/349 (84.5%)	0.042*
History of cat-bite wounds		256	3/20 (15%)	41/177 (23.2%)	0.574
Flea infestation		11	9/53 (17%)	80/389 (20.6%)	0.542
Tick infestation		12	0/52 (0%)	7/389 (1.8%)	1

<sup>†</sup> Non-stray category includes client-owned cats and cats lived in catteries

**Table 2.** Multivariate analysis of risk factors for seropositivity against feline coronavirus (FCoV) among 453 cats

<b>Variables</b>	<b>Categories</b>	<b>Odds Ratio</b>	<b>Confidence Interval</b>	<b>P value</b>
Cat acquisition	Non-stray†	Reference		
	Stray	2.94	1.26 - 8.03	0.019
Contact with other cats		4.54	1.32 - 28.57	0.042

† Non-stray category includes client-owned cats and cats lived in catteries

#### **4. Article No 3**

**Prospective epidemiological, clinical, and clinicopathologic evaluation of cats with *Bartonella* spp. and haemoplasma infections.** Submitted for publication



## **Original Article**

### **Prospective epidemiological, clinical, and clinicopathologic evaluation of cats with *Bartonella* spp. and haemoplasma infections**

## Abstract

The aim of this study was to describe the epidemiological, clinical, and clinicopathologic aspects of various populations of cats infected with *Bartonella* spp. or haemoplasma species. The populations evaluated included client-owned cats, stray cats and cats that live in catteries in Greece.

A total of 452 cats were prospectively enrolled into the study. A commercially available IFAT kit was used for the detection of *B. henselae* IgG antibodies in serum. PCRs for the detection of *Bartonella* spp. and haemoplasma species DNA in the blood were performed in a subgroup of 242 cats.

Overall, 160 (35.4%) of the 452 cats were seropositive for *B. henselae*. Seven (2.9%) and 46 (19%) of the 242 cats were PCR-positive for *Bartonella* spp. and haemoplasma species, respectively. Factors associated with *B. henselae*-seropositivity based on multivariate analysis, included higher body weight, stray or client-owned cats, outdoor access, higher number of in-contact cats, flea infestation, gingivostomatitis and hyperglobulinaemia. Factors associated with *Bartonella* spp. infection included living in rural areas, non-smooth kidney surface and hypercalcaemia. Factors associated with haemoplasma species infection included non-administration of ectoparasiticides, fever, ocular signs, exposure to *Toxoplasma gondii* and thrombocytopenia. Clinically healthy cats were more likely to be PCR-positive for haemoplasma species compared to sick cats.

This study shows a high prevalence of seropositivity for *B. henselae* and a relatively high prevalence of infection with haemoplasma species. Various clinical signs and laboratory abnormalities were identified to be significantly associated with exposure to and/or infection with *Bartonella* spp. and haemoplasma species.

**Keywords:** feline, molecular, prevalence, risk factors, serology, vector-borne

## Introduction

Bartonellosis and haemoplasmosis are vector-borne diseases with global impact on the health of domestic cats and of zoonotic importance.

*Bartonella* species are small, Gram-negative intracellular bacteria and cats are the main reservoir of *Bartonella henselae*, *B. clarridgeiae* and *B. koehlerae* and accidental hosts for *B. quintana*, *B. bovis* and *B. vinsonii* subsp. *berkhoffii*. The most common species infecting cats is *B. henselae*, which is the main causative agent of cat scratch disease in humans. The major mode of transmission among cats, is by exposure to flea bites (*Ctenocephalides felis*) or faeces, and uncommonly by aggressive interactions among cats (Bouhsira et al., 2013).

Haemoplasmas are haemotropic mycoplasmas that infect erythrocytes. The main haemoplasma species currently known to infect cats are *Mycoplasma haemofelis*, *Candidatus Mycoplasma haemominutum* and *Candidatus Mycoplasma turicensis*. Although the natural mode of transmission of haemoplasma species among cats is not clear, arthropod vectors, including *C. felis* and interaction with infected cats have been suggested (Sykes et al., 2007).

The reported serological and molecular prevalence of *Bartonella* spp. varies considerably, ranging between 0-85% and 0-83.5% (Bergh et al., 2002), respectively, depending on the geographical location and study population, while the molecular prevalence of haemoplasma species infection ranges between 4-43% (Attipa et al., 2017; Mifsud et al., 2020).

Cats naturally infected with *Bartonella* spp. and haemoplasma species may have chronic bacteraemia but no clinical signs. Uncommonly, cats naturally infected with *Bartonella* spp. may develop endocarditis, myocarditis, osteomyelitis, polyarthritis, uveitis and/or gingivostomatitis, while after natural infection with haemoplasma species they may develop fever, anorexia, weight loss, dehydration and/or splenomegaly. Various laboratory abnormalities have been reported in cats infected with *Bartonella* spp. or haemoplasma species, typically including anaemia, leukocytosis or leukopenia and hyperglobulinaemia (Ishak et al., 2007; Whittemore et al., 2012).

Only limited data are available on the epidemiology of *Bartonella* spp. and haemoplasma species in cats from Greece, and there is no study investigating the possible associations between infection and clinical or laboratory abnormalities in this country. Globally, limited prospective studies have been published to investigate both

risk factors (including, though not limited to, exposure to other common feline infectious agents) and the clinical and clinicopathologic findings of these two vector-borne pathogens in naturally infected cats.

The objectives of the present study were to: 1) determine the seroprevalence of *B. henselae* and the molecular prevalence of *Bartonella* spp. and haemoplasma species in different populations of cats living in different regions of Greece; 2) assess risk factors for seropositivity and/or PCR-positivity; 3) investigate possible associations between seropositivity for *B. henselae*, PCR-positivity for *Bartonella* spp. or PCR-positivity for haemoplasma species and seropositivity for other infectious agents, including *Toxoplasma gondii*, feline coronavirus (FCoV), feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV); 4) determine the clinical signs and haematological and biochemical findings associated with seropositivity and PCR-positivity for *Bartonella* spp. and PCR-positivity for haemoplasma species.

## **Materials and methods**

### *Ethics approval*

The study protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Thessaly (13/16-6-15). Handling of these animals was in compliance with the European Communities Council Directive 2010/63/EU and state laws.

### *Study population*

Cats from 4 different geographic areas of Greece (i.e., Attica, Thessaly, Crete and Macedonia) were prospectively enrolled by three clinicians between November 2013 and November 2016 and divided into three groups: client-owned cats, stray cats, and cats living in breeding catteries (Kokkinaki et al., 2021). Cats were presented by their owners (client-owned or cattery cats) or by two cat-rescue groups (stray cats) for wellness examination, vaccination, neutering and/or medical treatment. Stray cats often originated from cat-dense environments (i.e., rescue colonies) and their age was estimated based on body size, dentition and other physical characteristics.

Inclusion criteria for cats in all groups included: a) a body weight of  $\geq 0.5$  kg; and b) an informed consent form signed by the owner or the rescuer. Both healthy and diseased cats were randomly included into the study. Cats were considered healthy based on a normal physical examination and no recent history of disease. Cats

presented with systemic clinical (anorexia, depression, lethargy), gastrointestinal (vomiting, diarrhoea), ocular, neurological or dermatological signs (skin lesions and/or pruritus) were considered sick.

For the serological detection of *B. henselae* IgG antibodies, cats that fulfilled the above two criteria were enrolled on a sequential basis, regardless of their health status. For the molecular detection of *Bartonella* spp. and haemoplasma species, anaemic and non-anaemic cats were randomly selected from the initially enrolled cats at a 1:1 ratio, using a randomization table.

Signalment and historical data were collected using a standardized questionnaire and a thorough physical examination was performed for each cat by one of three authors (KGK, PGX, MEM). Historical information included: a) signalment; b) geographic origin and travel history; c) prior and current ownership; d) living conditions (indoors, outdoors); e) diet (dry food, canned food, home-cooked food, raw meat); f) vaccination status (fully vaccinated, partially vaccinated, unvaccinated); g) parasite prevention status (regular use of ectoparasiticides, intermittent use, non-use); h) prior medical problems; i) previous treatments; j) chief presenting complaint; k) present health status (e.g., weight loss, reduced appetite, faecal characteristics).

#### *Sample collection and laboratory analyses*

A total of 5 mL of blood was collected by jugular venipuncture from each cat. One mL of blood was sequentially transferred into two EDTA-anticoagulated tubes. One sample was used for haematology, while the other was stored at -80°C for PCR. The remaining blood was transferred in an anticoagulant-free tube, and serum was harvested following centrifugation at 3000 rpm for 20 min. Serum aliquots were stored at -80°C until biochemical analysis and indirect fluorescent antibody test (IFAT) were done.

Haematology was performed using one of three different haematology analyzers (Sysmex pocH-100i, ADVIA 2120i Siemens, ADVIA 120 Siemens), depending on the geographic area of sampling and the results were classified as normal, increased, or decreased, based on the reference intervals of each laboratory. For most cats, differential white blood cell count was calculated manually, and inspection for platelet aggregates was done in Diff-Quik (Merck, Germany) stained blood smears, whereas for the remaining cats (n=25) it was based on the results of analysis using the ADVIA 2120i analyzer. All haematologic analyses were performed

within 12 hours of blood collection. Biochemical analyses were performed using an automated chemistry analyzer (Roche/Hitachi MODULAR ANALYTICS D 2400 module, Roche Diagnostics, Switzerland, CH).

#### *Serologic testing*

*Bartonella henselae* IgG antibodies, *T. gondii* IgG antibodies and FCoV IgG antibodies were detected using commercially available IFAT kits (Biopronix Product Line, Agrilolabo S.p.a., Italy). The tests were performed according to manufacturer's instructions as previously described (Spada et al., 2016). Cut-off values were 1/64 for *B. henselae*, 1/50 for *T. gondii* and 1/100 for FCoV. All slides were examined by fluorescence microscopy at 40x magnification (Olympus, Japan)

Testing for FeLV antigen and FIV antibody was performed using an in-house ELISA assay (SNAP FIV/FeLV Combo test, IDEXX Laboratories, Westbrook, ME, USA) at the time of blood collection, using serum or plasma.

#### *Molecular analyses*

DNA was extracted from whole blood using the Nucleospin Tissue Kit (Macherey-Nagel GmbH & Co. KG, Germany), based on the instructions provided by the manufacturer and stored at -20°C until use.

The quality of the DNA was assessed for purity and integrity by submerged gel electrophoresis followed by image analysis using a Bio-Rad ChemiDoc XRS+ Molecular Imager (Bio-Rad Laboratories Inc., U.S.A.), and by spectrophotometry at 260/280 nm, using a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific Inc., U.S.A.). The presence of inhibitors in the samples was assessed by a PCR assay targeting a housekeeping gene (actin).

PCR was performed in an Applied Biosystems Verity 96-well Thermal Cycler (Thermo Fisher Scientific Inc., U.S.A.). Real-time PCR was performed in a Roche LightCycler 2.0 (Roche, Germany). PCR products were analyzed by submerged electrophoresis using 2% agarose gels stained with ethidium bromide (0.5 µg/mL) and visualized using a Bio-Rad ChemiDoc XRS+ Molecular Imager (Bio-Rad Laboratories Inc., U.S.A.). For confirmation of the specificity of the amplification products, approximately 20% of the PCR products were submitted for sequence analysis, which was conducted on both strands using the Applied Biosystems BigDye Terminator Cycle Sequencing Kit and PRISM 377 DNA Sequencer (Thermo Fisher

Scientific Inc., U.S.A.). Results were analyzed and compared to deposited sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI).

#### *PCR protocol for detection of Bartonella spp.*

The target sequence was a 209 base-pair fragment of the *Bartonella* *ssrA* gene (Forward: GCTATGGTAATAAATGGACAATGAAATAA, Reverse: GACGTGCTTCCGCATAGTTGTC) (Mylonakis et al., 2018). A 25 µl mixture for each PCR reaction was performed with the Kapa Taq PCR kit following the standard protocol suggested by the manufacturer. The amplification profile consisted of an initial denaturation step at 95°C for 5 min followed by 30 cycles of incubation at 95°C for 30 sec, 60°C for 45 sec and a final elongation step at 72°C for 5 min.

#### *PCR protocol for detection of Mycoplasma spp.*

The target sequence was a fragment of the *Mycoplasma* 16S-rRNA gene (Forward: ACGAAAGTCTGATGGAGCAATA, Reverse: ACGCCCAATAAATCCGRATAAT) (Jensen et al., 2001). A 25 µl mixture for each PCR reaction was performed with the Kapa Taq PCR kit, following the standard protocol suggested by the manufacturer. A hot-start PCR was performed, starting with 5 min at 95°C. The amplification profile consisted of an initial denaturation step at 95°C for 5 min followed by 30 cycles of incubation at 95°C for 30 sec, 60°C for 45 sec and a final elongation step at 72°C for 5 min.

#### *Statistical analysis*

For univariate analysis, categorical data regarding signalment, historical information, clinical signs, haematology and biochemistry in *Bartonella* spp. or haemoplasma species PCR-positive cats were compared to those of *Bartonella* spp. or haemoplasma species PCR-negative cats by either Pearson's  $\chi^2$  or Fisher's exact test. The same variables were compared between *B. henselae* seropositive and seronegative cats. The normality of the distribution of the continuous variables was tested using the Kolmogorov-Smirnov test. Normally distributed data are presented as means  $\pm$  standard deviation and were compared between PCR-positive or seropositive cats and PCR-negative or seronegative cats using independent sample t-tests. Not normally distributed data are presented as medians and ranges and were compared

between PCR-positive or seropositive cats and PCR-negative or seronegative cats using Mann Whitney *U* tests.

Variables that were different at 25% level of significance, between *Bartonella* spp. or haemoplasma species PCR-positive and PCR-negative cats in the univariate analysis were selected as candidates for 3 initial logistic regression models. The first model included significant signalment and history variables, the second model included significant clinical examination and testing for other agents variables and the third model included significant haematology and biochemistry variables. These 3 initial models were subsequently reduced in a stepwise manner until only significant at  $P < 0.05$  variables remained.

Similarly, variables that were different at  $P < 0.25$  between *B. henselae* seropositive and seronegative cats in the univariate analysis were selected as candidates for 4 initial logistic regression models relating to: a) signalment and history variables, b) clinical examination and testing for other infectious agents variables, c) haematology variables and d) biochemistry variables. The initial models were subsequently reduced in a stepwise manner until only significant at  $P < 0.05$  variables remained. Odds ratios (OR) derived from the reduced models were interpreted as measures of increased risk of seropositivity or PCR-positivity. The analyses were done using Stata 13 (Stata Corp, College Station, TX) and SPSS 23 for Windows (IBM Corp, Armonk, NY).

## Results

### *Serology*

A total of 452 cats were enrolled. The age of the cats ranged from 6 weeks to 17 years (median 2 years). Two hundred and forty-one cats (53.3%) were males (86 neutered; 35.7% of the male cats) and 211 (46.7%) were females (52 neutered; 24.6% of the female cats). Fifteen cats (3.3%) were purebred and 422 (93.4%) were common European cats, while for 15 (3.3%) cats the breed was not recorded. Ninety-seven cats (21.5%) lived exclusively indoors and 333 (73.7%) outdoors (exclusively or partially), while for 22 cats (4.9%) the status was unknown. Two hundred and fifty-five cats (56.4%) lived in Attica, 79 (17.5%) in Crete, 73 (16.2%) in Thessaly and 45 (10%) in Macedonia. Two hundred and sixty-seven cats (59.1%) were client-owned, 155 (34.3%) were stray and 21 (4.6%) lived in catteries, while for 9 cats (2%) these data were missing. Three hundred and sixty-one cats (79.9%) lived in urban areas and



68 (15%) in rural areas, while for 23 (5.1%) the area was unknown. One hundred and sixty-seven cats (36.9%) were clinically healthy and 274 (60.6%) were sick, while the health status was unknown for 11 cats (2.4%).

Of the 452 cats, 160 (35.4%) were found to be seropositive for *B. henselae*. Univariate associations between seropositivity for *B. henselae* and signalment-historical data, clinical signs, results of haematology and serum biochemistry results are presented in supplementary Tables S1, S2, S3, S4.

Several factors were found to be significantly associated with *B. henselae*-seropositivity in the multivariate analysis (Table 1). Seropositive cats weighed significantly more than seronegative cats. In addition, a higher number of in-contact cats and flea infestation were risk factors for seropositivity. Stray and client-owned cats were significantly more likely to be seropositive than cats living in catteries; also, cats living outdoors were significantly more likely to be seropositive than cats living indoors. Gingivostomatitis was also significantly associated with seropositivity. Cats with hyperglobulinaemia were more likely to be seropositive compared to cats with a normal or decreased serum globulin concentration, while cats with an increased serum phosphorus concentration were significantly less likely to be *B. henselae*-seropositive compared to cats with a normal serum phosphorus concentration.

### PCR

A total of 242 cats were selected for molecular detection of *Bartonella* spp. and *Mycoplasma* spp. infections. The age of these cats ranged from 6 weeks to 15 years (median 1 year). One hundred and thirty cats (53.7%) were male (49 neutered) and 112 (46.3%) were female (22 neutered). Two cats (0.8%) were purebred and 234 (96.7%) were common European cats, while for 6 (2.5%) cats the breed was not recorded. Thirty cats (12.4%) lived exclusively indoors and 205 (84.7%) outdoors (exclusively or partially), while for 7 cats (2.9%) the status was unknown. Ninety-four cats (38.8%) lived in Attica, 70 (28.9%) in Crete, 60 (24.8%) in Thessaly, and 18 (7.4%) in Macedonia. One hundred and thirty-seven cats (56.6%) were client-owned, 91 (37.6%) were stray and 12 (5%) were living in catteries, while for 2 cats (0.8%) these data were missing. One hundred and seventy-six cats (72.7%) lived in urban areas and 58 (24%) in rural areas, while for 8 cats (3.3%) the area was unknown. Seventy-four cats (30.6%) were clinically healthy and 165 (68.2%) were sick, while the health status was unknown for 3 cats (1.2%).

Of the 242 cats, a total of 7 (2.9%) and 46 (19%) were found to be PCR-positive for *Bartonella* spp. and haemoplasma species, respectively. Of the PCR-positive cats, 3 (1.2% of the total 242 cats) had concurrent PCR-positive results for *Bartonella* spp. and haemoplasma species. The univariate associations between PCR-positivity for *Bartonella* spp. (Tables S5, S6, S7) or haemoplasma species (Tables S8, S9, S10) and signalment-historical data, clinical signs, results of serology, haematology and serum biochemistry are presented in supplementary files.

Factors significantly associated with PCR-positivity for *Bartonella* spp. in the multivariate analysis are presented in Table 2. Cats living in rural areas were significantly more likely to be positive than cats living in urban areas. Non-smooth kidney surface was also significantly associated with PCR-positivity for *Bartonella* spp. In addition, cats with hypercalcemia were more likely to be *Bartonella* spp.-positive compared to cats with normal or reduced serum calcium concentrations.

Multivariate analysis also indicated several factors associated with PCR-positivity for *Mycoplasma* spp. (Table 3). Clinically healthy cats and cats who had not been treated with a preventative ectoparasiticide were significantly more likely to be haemoplasma species-positive than sick cats and cats who had been treated with ectoparasiticides, respectively. The presence of fever and ocular signs was also significantly associated with PCR-positivity for haemoplasma species. In addition, seropositivity for *Toxoplasma gondii* was associated with an increased risk for PCR-positivity for haemoplasma species. Finally, cats with thrombocytopenia were significantly more likely to be PCR-positive for haemoplasma species compared to cats with a normal platelet count.

## Discussion

This is one of the largest prospective studies on natural infection with *Bartonella* spp. and *haemoplasma* species in cats. Overall, 35.4% of the cats were seropositive for *B. henselae*, while 2.9% and 19% of the cats were PCR-positive for *Bartonella* spp. and haemoplasma species, respectively. Flea infestation, access to outdoor or multi-cat environments, gingivostomatitis and hyperglobulinaemia were significant predictors of *B. henselae*-seropositivity. Non-smooth kidney surface and hypercalcemia were associated with PCR-positivity for *Bartonella* spp., while fever, ocular signs, seropositivity to *T. gondii* and thrombocytopenia were associated with PCR-positivity for haemoplasma species.

The haemoplasma species prevalence by PCR identified here is similar to results from a previous prospective study in cats from Greece, where the prevalence was reported to be 20.6% (Maher et al., 2010). However, in another study in cats from Greece, the authors reported a seroprevalence of *B. henselae* of only 4% (Chatzis et al., 2014). More recently, in a retrospective study of 100 cats from Greece, 8.5% and 14.9% were PCR-positive for *Bartonella* spp. and haemoplasma species, respectively (Mylonakis et al., 2018). Seroprevalence for *Bartonella* spp. in cats has been reported to be 0%-68% in Europe (Mietze et al., 2011), 0%-85.2% in the USA (Jameson et al., 1995), 37% in Australia (Barrs et al., 2010) and 11%-59% in Africa (Tiao et al., 2013). Studies have reported an overall PCR-based prevalence of feline infection with haemoplasma species, of 32.1% in South Africa (Lobetti and Tasker, 2004), 27.2% in Australia (Tasker et al., 2004), 26.4% in Asia (Tanahara et al., 2010), 12%-27% in the USA (Sykes et al., 2008), and 4%-43% in Europe (Mifsud et al., 2020). The variability of serologic and molecular prevalence among these studies may reflect differences in the demographics and the geographic origin of the cats, in the accuracy of the diagnostic tests and/or the overtime changes in the prevalence of these infections.

Our study showed that stray cats and cats with outdoor access were more likely to be *B. henselae*-seropositive compared to cats living in catteries and cats living strictly indoors, respectively and this is in agreement with previous reports likely reflecting increased access to flea-infested environments (Guptill et al., 2004). Interestingly, client-owned cats were more likely to be seropositive than cats living in catteries. Although the reason for this is not known, it may partially reflect on the high awareness amongst cat breeders of the risks of vector-borne infections and the implementation of strict flea control measures.

A significant association was established between *Bartonella* spp. seropositivity and the number of in-contact cats. These cats are expected to have a higher prevalence of *B. henselae*-seropositivity, as they are exposed to more cats and pathogen vectors. This finding is in accordance with the results of a previous study in 436 client-owned cats in France, where the prevalence of bacteremia and seropositivity increased as the number of cats increased (Gurfield et al., 2001).

Cats infested with fleas were more likely to be *B. henselae*-seropositive compared to cats without flea infestation. Many studies have documented that *B. henselae* is naturally transmitted among cats by the flea *C. felis* or by flea faeces

(Bouhsira et al., 2013). *C. felis* is the major flea species affecting cats in Greece (Koutinas et al., 1995).

Body weight was significantly higher in *B. henselae*-seropositive cats compared to seronegative cats. However, because body weight increases during growth and because univariate analysis showed that older age was associated with increased risk of *B. henselae*-seropositivity, this finding most likely reflects the older age of seropositive cats.

Our study indicates a significant association between *B. henselae*-seropositivity and gingivostomatitis. However, conflicting data exist regarding associations between gingivostomatitis and *Bartonella* spp. bacteraemia and/or seropositivity. In two studies of naturally infected cats, an association between seropositivity and stomatitis was suggested (Ueno et al., 1996; Glaus et al., 1997), while in another study an association was reported between *Bartonella* spp. bacteraemia and oral disease, but not between seropositivity and oral disease (Sykes et al., 2010). The association between gingivostomatitis and *Bartonella* spp. requires further investigation.

Similar to other studies, we found that *B. henselae*-seropositive cats had a significantly increased risk of hyperglobulinaemia (Ishak et al., 2007; Whittemore et al., 2012). Hyperglobulinaemia in *Bartonella* spp.-seropositive cats is typically a polyclonal gammopathy due to the prolonged bacteraemia (Whittemore et al., 2012).

Cats with an increased serum phosphorus concentration were significantly more likely to be *B. henselae*-seronegative than cats with a normal serum phosphorus concentration. Because serum concentration of phosphorus is increased in young healthy animals, this finding most likely reflects the younger age of *B. henselae*-seronegative cats found in the univariate analysis.

Cats living in rural areas were more likely to be PCR-positive for *Bartonella* spp. than cats living in urban areas. This is consistent with a recent study, where a significant association was found between infection with feline vector-borne pathogens, including *B. henselae*, and a rural habitat (Attipa et al., 2017), likely because *C. felis* is more prevalent in rural habitats.

A non-smooth surface of the kidneys on abdominal palpation was more prevalent in *Bartonella* spp.-PCR positive compared to PCR-negative cats. This has not been previously reported and should be interpreted with caution as this was seen in a single PCR-positive cat. Experimentally-induced feline bartonellosis causes

granulomatous inflammation of the kidneys, which may account for the kidney surface distortion (Kordick et al., 1999). However, in our study the single positive cat had concurrent infections with FeLV and FIV and was seropositive for FCoV. Therefore, this clinical finding may not be associated with bartonellosis but rather with infection with these concomitant infections.

Hypercalcaemic cats were significantly more likely to be *Bartonella* spp. PCR-positive than cats with a normal serum calcium concentration. Again, this finding should be interpreted cautiously as only one PCR-positive cat had hypercalcaemia. Due to the fact that this cat also had an increased serum creatinine concentration, hypercalcemia was most likely associated with chronic kidney disease. Also, idiopathic hypercalcaemia, which is an important differential diagnosis in cats, cannot be ruled out.

Cats that had not been on a preventative ectoparasiticide were more likely to be PCR-positive for haemoplasmas than cats previously treated with an ectoparasiticide. It is known that haemoplasmas are mainly transmitted through arthropod vectors and *C. felis* has been implicated in feline haemoplasma transmission (Woods et al., 2003). Also, ticks have been proposed as potential vectors since feline haemoplasma species have been found in ticks collected from cats (Duplan et al., 2018).

Fever was also found to be significantly associated with haemoplasma species PCR-positivity and this is consistent with the results of another study, where an association between *M. haemofelis* and fever in cats without anaemia was found (Lappin et al., 2020). In a recent retrospective study, the prevalence of haemoplasma infection in 106 cats with pyrexia was 7.5% (Spencer et al., 2017). In addition, a study of 22 anaemic cats naturally infected with haemoplasma species, reported that 10% of those cats had a fever (Weingart et al., 2016).

Ocular signs were significantly associated with haemoplasma species PCR-positivity and this is the first time such an association has been reported. In our study, exposure to *T. gondii* was significantly associated with haemoplasma infection. It is well documented that the eye is the most commonly affected organ in feline toxoplasmosis. Furthermore, in our study all haemoplasma species-positive cats with ocular signs were affected by upper respiratory track disease (URTD) that is commonly accompanied by ocular discharge and lesions. Therefore this association

was most likely due to *T. gondii* infection or URTD and less likely due to haemoplasmosis.

Another finding of our study was the higher prevalence of haemoplasma species PCR-positivity among clinically healthy cats compared to sick cats. This finding could reflect the fact that the group of sick cats was quite inhomogeneous. There were cats with severe systemic clinical signs but also cats with skin lesions, reproductive and musculoskeletal disorders or neurological and ocular signs. This fact could have influenced the results. Indeed, when only sick cats with compatible clinical signs of mycoplasmosis were compared with clinically healthy cats, no statistically significant difference was identified.

Seropositivity for *T. gondii* was found to be significantly associated to haemoplasma species PCR-positivity. Although the natural mode of transmission of haemoplasma infection has not been clearly established, one possible explanation could be a similar mode of transmission of the two agents. Similarly to congenital infection with *Toxoplasma gondii*, it has been suggested that *M. haemofelis* can be transmitted from queens with clinical disease to their kittens (Fisher et al., 1983).

Thrombocytopenia was found to be associated with haemoplasma species PCR-positivity. In a retrospective study in 102 cats from South Africa, the prevalence of thrombocytopenia was significantly higher in *M. haemofelis*-infected cats than in non-infected cats (Lobetti and Lappin, 2012). Similarly, in a subsequent study of naturally haemoplasma-infected cats from Brazil, thrombocytopenia was statistically associated with infection by *M. haemofelis* (Raimundo et al., 2016). In our study, low platelet count was confirmed by blood smear quantification of platelets in 26/30 sick cats with thrombocytopenia.

A limitation of our study is that some cats had not undergone an extensive diagnostic investigation to establish the underlying cause(s) of their illness. In addition, the lack of identification of *Bartonella* spp. and haemoplasmas on a species level in this study, prevented the potential expansion of the spectrum of these agents affecting felines in Greece and the investigation of the clinical and clinicopathologic relevance of different species of *Bartonella* and haemoplasmas.

## Conclusion

This study showed a high prevalence of seropositivity for *B. henselae* and a relatively high prevalence of haemoplasma species infection. Stray cats, outdoor

access, multiple in-contact cats and flea infestation were identified as risk factors for *B. henselae* seropositivity. Living in rural areas and lack of ectoparasiticide use were identified as risk factors for *Bartonella* spp. and haemoplasma species PCR-positivity, respectively. Gingivostomatitis and hyperglobulinaemia were associated with *B. henselae*-seropositivity. Clinically healthy cats were more likely to be haemoplasma species PCR-positive, while fever, exposure to *T. gondii* and thrombocytopenia were associated with haemoplasma species PCR-positivity.

Future prospective studies in naturally infected cats are warranted in order to investigate the clinical and clinicopathologic relevance of different species of *Bartonella* and haemoplasmas in cats in Greece and other geographic locations.

### **Conflict of interest statement**

IDEXX Laboratories and Petline supplied the SNAP FIV/FeLV Combo tests used in this study. IDEXX Laboratories and Petline did not participate in the study design, sample collection, analysis, interpretation of data, or in the decision to submit the manuscript for publication. None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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**Table 1.** Multivariate analysis of signalment, historical data, clinical signs, and the results of haematology and serum biochemistry identified as factors associated with *Bartonella henselae* seropositivity

Variables	Categories	Seropositivity (%)	OR	CI	<i>P value</i>
Body weight (kg)			1.42	1.18 - 1.7	< 0.001
Current ownership	Cattery	3/21 (14.3%)	Reference		
	Stray	60/155 (38.7%)	8.38	1.04 - 67.42	0.04
	Client-owned	94/267 (35.2%)	12.47	1.56 - 99.47	0.01
Living conditions	Indoors	21/97 (21.6%)	Reference		
	Outdoors	129/333 (38.7%)	2.47	1.15 - 5.32	0.02
Number of in-contact cats			1.45	1.07 - 1.97	0.01
Flea infestation	No	113/354 (31.9%)	Reference		
	Yes	43/87 (49.4%)	2	1.05 – 3.82	0.03
Gingivostomatitis	No	122/380 (32.1%)	Reference		
	Yes	34/64 (53.1%)	2.39	1.4 - 4.09	0.001
Globulins	Normal	84/281 (29.9%)	Reference		
	Increased	74/140 (52.8%)	2.38	1.55 - 3.65	< 0.001
Globulins	Decreased	1/19 (5.2%)	Reference		
	Increased	74/140 (52.8%)	14.09	1.79 - 111.02	0.012
Inorganic phosphorus	Normal	97/224 (43.3%)	Reference		
	Increased	61/213 (28.6%)	0.56	0.36 - 0.87	0.01

OR: odds ratio; CI: confidence interval

**Table 2.** Multivariate analysis of historical data, clinical signs, and results of haematology and serum biochemistry, identified as factors associated with *Bartonella* spp. PCR-positive status

Variables	Categories	Positive cats (%)	OR	CI	<i>P value</i>
Living area	Urban	2/176 (1.1%)	Reference		
	Rural	5/58 (8.6%)	8.2	1.54-43.53	0.01
Non-smooth kidney surface	No	6/238 (2.5%)	Reference		
	Yes	1/3 (33.3%)*	19.33	1.53-243.56	0.02
Calcium	Normal	6/218 (2.7%)	Reference		
	Increased	1/3 (33.3%)**	18.5	1.46-233.11	0.024

OR: odds ratio; CI: confidence interval

\* The single *Bartonella* spp. PCR-positive cat with irregular kidney surface had concurrent infections with FeLV and FIV and was seropositive for FCoV

\*\* The single *Bartonella* spp. PCR-positive cat with hypercalcemia had also increased serum creatinine

**Table 3.** Multivariate analysis of historical data, clinical signs and results of serology and haematology identified as factors associated with *Mycoplasma* spp. PCR-positive status

Variables	Categories	Positive cats (%)	OR	CI	<i>P value</i>
Use of ectoparasiticides		8/83 (9.6%)	0.29	0.12 - 0.72	0.007
Fever		3/6 (50%)	6.85	1.2 - 39.12	0.03
Ocular signs		13/50 (26%)*	3.14	1.27 - 7.75	0.013
At least one clinical sign		28/165 (16.9%)	0.42	0.18 - 0.96	0.04
<i>Toxoplasma gondii</i> IgG antibodies		17/53 (32%)	3.18	1.48 - 6.82	0.003
Platelets	Normal	27/176 (15.3%)	Reference		
	Decreased	16/49 (32.6%)	2.67	1.29 - 3.22	0.008

OR: odds ratio; CI: confidence interval

\*All haemoplasma species-positive cats with ocular signs were affected by upper respiratory track disease (URTD)

**Supplementary data-Table S1.** Univariable associations between *Bartonella henselae* seropositivity and the signalment and historical data that were collected using a standardized questionnaire. Variables that were used in the logistic regression model ( $P$  value  $< 0.25$ ) are highlighted with an asterisk

Variables	Categories	Missing data	<i>Bartonella henselae</i> IgG antibodies		
			Seropositive (%)	Seronegative (%)	$P$ value
Sex	Male	0	78/160 (48.8%)	163/292 (55.8%)	0.15*
	Female		82/160 (51.2%)	129/292 (44.2%)	
Neutered		6	49/157 (31.2%)	89/289 (30.8%)	0.928
Breed	Purebred	15	2/154 (1.3%)	13/283 (4.6%)	0.071*
	Common European breed		152/154 (98.7%)	270/283 (95.4%)	
Age (years)		23	2 (0.13-17)	1 (0.13-15)	<0.001*
Body weight (kg)		45	3.4 (0.5-8)	3.15 (0.5-9)	0.014*
Cat acquisition	Stray	93	87/118 (73.7%)	170/241 (70.5%)	0.529
	Non-stray		31/118 (26.3%)	71/241 (29.5%)	
Current ownership	Client-owned	9	94/157 (59.9%)	173/286 (60.5%)	0.081*
	Stray		60/157 (38.2%)	95/286 (33.2%)	
	Cattery		3/157 (1.9%)	18/286 (6.3%)	
Living conditions	Indoors	22	21/150 (14%)	76/280 (27.1%)	0.002*
	Outdoors		129/150 (86%)	204/280 (72.9%)	
Living area	Urban	23	120/149 (80.5%)	241/280 (86.1%)	0.135*
	Rural		29/149 (19.5%)	39/280 (13.9%)	
Contact with other cats		57	131/141 (92.9%)	208/254 (81.8%)	0.003*
Number of in-contact cats	1-2 cats	127	18/112 (16.1%)	38/213 (17.8%)	0.01*
	3 cats		5/112 (4.5%)	6/213 (2.8%)	
	>3 cats		79/112 (70.5%)	121/213 (56.8%)	
History of cat-bite wounds		253	15/55 (27.3%)	31/144 (21.5%)	0.39
Use of ectoparasiticides		187	56/91 (61.5%)	101/174 (58%)	0.583
Flea infestation		11	43/156 (27.6%)	44/285 (15.4%)	0.002*

Tick infestation	12	5/156 (3.2%)	2/284 (0.7%)	0.103*
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**Supplementary data-Table S2.** Univariable associations between *Bartonella henselae* seropositivity, the presence of clinical signs, the results of PCR for *Bartonella* spp. and for *Mycoplasma* spp. and the results of serology for *Toxoplasma gondii*, feline coronavirus, feline leukemia virus and feline immunodeficiency virus. Variables with that were used in the logistic regression model (P value <0.25) are highlighted with an asterisk

Clinical sign	Missing data	<i>Bartonella henselae</i> IgG antibodies		
		Seropositive (%)	Seronegative (%)	P value
Weight loss	128	11/102 (10.8%)	32/222 (14.4%)	0.371
Anorexia (previous 3 weeks)	96	16/117 (13.7%)	47/239 (19.7%)	0.164*
Depression-lethargy	8	16/156 (10.3%)	33/288 (11.5%)	0.7
Weakness	8	4/156 (2.6%)	6/288 (2.1%)	0.746
Dehydration	10	10/155 (6.5%)	22/287 (7.7%)	0.638
Fever	12	3/153 (2%)	9/287 (3.1%)	0.555
Lymphadenomegaly	10	8/155 (5.2%)	15/287 (5.2%)	0.977
Abnormal lung sounds	10	1/155 (0.6%)	9/287 (3.1%)	0.176*
Cough	5	2/158 (1.3%)	5/289 (1.7%)	1
Dyspnea	9	2/155 (1.3%)	2/288 (0.7%)	0.614
Signs of rhinitis	9	12/156 (7.7%)	25/287 (8.7%)	0.711
Ocular signs	18	13/153 (8.5%)	38/281 (13.5%)	0.12*
Gingivostomatitis	8	34/156 (21.8%)	30/288 (10.4%)	0.001*
Vomiting	130	4/100 (4%)	15/222 (6.8%)	0.331
Diarrhea	135	2/98 (2%)	14/219 (6.4%)	0.163*
Constipation	146	1/92 (1.1%)	1/214 (0.5%)	0.512
Abdominal pain	8	2/156 (1.3%)	6/288 (2.1%)	0.719
Abdominal mass	10	1/156 (0.6%)	1/286 (0.3%)	1
Abdominal effusion	8	2/156 (1.3%)	4/288 (1.4%)	1
Splenomegaly	9	0/156 (0%)	2/287 (0.7%)	0.543
Decreased kidney size	9	1/156 (0.6%)	1/287 (0.3%)	1
Increased kidney size		2/156 (1.3%)	3/287 (1%)	
Non-smooth kidney surface	9	3/156 (1.9%)	1/287 (0.3%)	0.128*
Polyuria-polydipsia	150	0/88 (0%)	4/214 (1.9%)	0.326
Dysuria	145	0/90 (0%)	5/217 (2.3%)	0.326

Pyometra or penile swelling	8	0/156 (0%)	3/288 (1%)	0.555
Skin lesions and/or pruritus	14	40/155 (25.8%)	54/283 (19.1%)	0.101*
Neurological signs	9	7/156 (4.5%)	10/287 (3.5%)	0.6
Musculoskeletal signs	8	4/156 (2.6%)	14/288 (4.9%)	0.241*
At least one clinical sign	11	97/155 (62.6%)	177/286 (61.9%)	0.886
At least one clinical sign compatible with bartonellosis	11	48/155 (31%)	77/286 (26.9%)	0.368
<i>Bartonella</i> spp. infection (PCR)	228	3/81 (3.7%)	4/143 (2.8%)	0.705
<i>Mycoplasma</i> spp. infection (PCR)	228	18/81 (22.2%)	26/143 (18.2%)	0.465
<i>Toxoplasma gondii</i> IgG antibodies (IFAT)	0	46/160 (28.7%)	48/292 (16.4%)	0.002*
Feline coronavirus IgG antibodies (IFAT)	3	23/159 (14.5%)	31/290 (10.7%)	0.239*
Feline leukemia virus infection	91	5/121 (4.1%)	5/240 (2.1%)	0.313
Feline immunodeficiency virus infection	91	14/121 (11.6%)	19/240 (7.9%)	0.256*

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IFAT: immunofluorescence antibody test; PCR: polymerase chain reaction

**Supplementary data-Table S3.** Univariable associations between *Bartonella henselae* seropositivity and the abnormal results of haematology. Variables that were used in the logistic regression model ( $P$  value  $<0.25$ ) are highlighted with an asterisk

Variable	Categories	Missing data	<i>Bartonella henselae</i> IgG antibodies		
			Seropositive (%)	Seronegative (%)	$P$ value
HCT	Decreased	0	33/138 (23.9%)	76/231 (32.9%)	0.067*
White blood cells	Decreased		6/138 (4.3%)	15/231 (6.5%)	0.156*
White blood cells	Increased	0	34/138 (24.6%)	75/231 (32.5%)	
Neutrophils	Decreased		13/138 (9.4%)	24/231 (10.4%)	0.983
Neutrophils	Increased	0	22/138 (15.9%)	37/231 (16%)	
Lymphocytes	Decreased		17/138 (12.3%)	31/231 (13.4%)	0.498
Lymphocytes	Increased	0	9/138 (6.5%)	23/231 (10%)	
Monocytes	Increased	0	4/138 (2.9%)	11/231 (4.8%)	0.38
Eosinophils	Decreased		7/138 (5.1%)	4/231 (1.7%)	0.086*
Eosinophils	Increased	0	42/138 (30.4%)	59/231 (25.5%)	
Platelets	Decreased		26/138 (18.8%)	50/231 (21.6%)	0.06*
Platelets	Increased	0	3/138 (2.2%)	17/231 (7.4%)	

HCT: haematocrit

**Supplementary data-Table S4.** Univariable associations between *Bartonella henselae* seropositivity and the abnormal results of serum biochemistry. Variables that were used in the logistic regression model ( $P$  value  $<0.25$ ) are highlighted with an asterisk

Variable	Categories	Missing data	<i>Bartonella henselae</i> IgG antibodies		
			Seropositive (%)	Seronegative (%)	$P$ value
Total proteins	Decreased	12	7/159 (4.4%)	33/281 (11.7%)	$<0.001^*$
Total proteins	Increased		14/159 (8.8%)	5/281 (1.8%)	
Albumins	Decreased	8	124/160 (77.5%)	210/284 (73.9%)	0.405
Globulins	Decreased	12	1/159 (0.6%)	18/281 (6.4%)	$<0.001^*$
Globulins	Increased		74/159 (46.5%)	66/281 (23.5%)	
Urea nitrogen	Decreased	15	18/158 (11.4%)	40/280 (14.3%)	0.689
Urea nitrogen	Increased		10/158 (6.3%)	19/280 (6.8%)	
Creatinine	Decreased	10	9/159 (5.7%)	41/283 (14.5%)	0.013 <sup>*</sup>
Creatinine	Increased		6/159 (3.8%)	7/283 (2.5%)	
Glucose	Decreased	15	7/158 (4.4%)	13/280 (4.6%)	0.756
Glucose	Increased		91/158 (57.6%)	151/280 (53.9%)	
Cholesterol	Decreased	16	20/158 (12.7%)	22/279 (7.9%)	0.231 <sup>*</sup>
Cholesterol	Increased		1/158 (0.6%)	3/279 (1.1%)	
Triglycerides	Decreased	12	3/159 (1.9%)	4/281 (1.4%)	0.431
Triglycerides	Increased		10/159 (6.3%)	11/281 (3.9%)	
Total bilirubin	Increased	9	6/159 (3.8%)	8/284 (2.8%)	0.581
ALP	Increased	8	10/160 (6.3%)	30/284 (10.6%)	0.127 <sup>*</sup>
ALT	Increased	16	3/155 (1.9%)	11/281 (3.9%)	0.396
AST	Increased	8	18/160 (11.3%)	29/284 (10.2%)	0.733
Calcium	Decreased	8	8/160 (5%)	10/284 (3.5%)	0.568
Calcium	Increased		2/160 (1.3%)	2/284 (0.7%)	
Inorganic phosphorus	Increased	16	61/158 (38.6%)	152/279 (54.5%)	0.001 <sup>*</sup>
Potassium	Decreased	10	3/160 (1.9%)	2/282 (0.7%)	0.364
Potassium	Increased		39/160 (24.4%)	80/282 (28.4%)	
Sodium	Decreased	9	19/160 (11.9%)	30/283 (10.6%)	0.49
Sodium	Increased		13/160 (8.1%)	33/183 (11.7%)	
Chloride	Decreased	9	56/160 (35%)	89/283 (31.4%)	0.34

Chloride	Increased	1/160 (0.6%)	7/283 (2.5%)
ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase			

**Supplementary data-Table S5.** Univariable associations between the infection status of 242 cats tested for *Bartonella* spp. by PCR and the signalment and historical data that were collected using a standardized questionnaire. Variables that were used in the logistic regression model (P value <0.25) are highlighted with an asterisk

Variables	Categories	Missing data	<i>Bartonella</i> spp. PCR-positive status		P value
			Positive (%)	Negative (%)	
Sex	Male	0	4/7 (57.1%)	126/235 (53.6%)	1
	Female		3/7 (42.9%)	109/235 (46.5%)	
Neutered		3	1/7 (14.3%)	70/232 (30.2%)	0.677
Breed	Purebred	6	0/7 (0%)	2/229 (0.9%)	1
	Common European Breed		7/7 (100%)	227/229 (99.1%)	
Age (years)		9	0.42 (0.33-3.5)	1 (0.13-15)	0.338
Body weight (kg)		19	2.1 (1.5-3.8)	3 (0.5-8)	0.365
Cat acquisition	Stray	58	1/6 (16.7%)	111/178 (62.4%)	0.035*
	Non-stray		5/6 (83.3%)	67/178 (37.6%)	
Current ownership	Stray	2	2/7 (28.6%)	89/233 (38.2%)	0.712
	Non-stray		5/7 (71.4%)	144/233 (61.8%)	
Living conditions	Indoors	7	0/7 (0%)	30/228 (13.2%)	0.6
	Outdoors		7/7 (100%)	198/228 (86.8%)	
Living area	Urban	8	2/7 (28.6%)	174/227 (76.7%)	0.011*
	Rural		5/7 (71.4%)	53/227 (23.3%)	
Contact with other cats		11	7/7 (100%)	210/224 (93.8%)	1
Number of in-contact cats	≤ 2 cats	56	0/6 (0%)	34/180 (18.9%)	0.594
	> 2 cats		6/6 (100%)	146/180 (81.1%)	
History of cat-bite wounds		120	0/5 (0%)	28/117 (23.9%)	0.588
Flea infestation		6	1/7 (14.3%)	62/229 (27.1%)	0.678
Tick infestation		7	0/7 (0%)	5/228 (2.2%)	1

PCR: polymerase chain reaction

**Supplementary data-Table S6.** Univariable associations between the infection status of 242 cats tested for *Bartonella* spp. by PCR and the presence of clinical signs, the results of serology for *Bartonella henselae* and of PCR for *Mycoplasma* spp. and the results of serology for *Toxoplasma gondii*, feline coronavirus, feline leukemia virus and feline immunodeficiency virus. Variables that were used in the logistic regression model (P value <0.25) are highlighted with an asterisk

Clinical sign	Missing data	<i>Bartonella</i> spp. PCR status		P value
		Positive (%)	Negative (%)	
Weight loss	76	0/5 (0%)	32/161 (19.9%)	0.584
Anorexia (previous 3 weeks)	66	1/6 (16.7%)	31/170 (18.2%)	1
Depression-lethargy	1	1/7 (14.3%)	29/234 (12.4%)	1
Weakness	1	0/7 (0%)	6/234 (2.6%)	1
Dehydration	2	1/7 (14.3%)	17/233 (7.3%)	0.425
Fever	5	1/7 (14.3%)	5/230 (2.2%)	0.166*
Lymphadenomegaly	3	0/7 (0%)	11/232 (4.7%)	1
Abnormal lung sounds	3	0/7 (0%)	11/232 (4.7%)	1
Cough	0	0/7 (0%)	8/235 (3.4%)	1
Dyspnea	2	0/7 (0%)	3/233 (1.3%)	1
Signs of rhinitis	2	0/7 (0%)	31/233 (13.3%)	0.599
Ocular signs	5	0/7 (0%)	50/230 (21.7%)	0.35
Gingivostomatitis	1	0/7 (0%)	38/234 (16.2%)	0.6
Glossitis	1	1/7 (14.3%)	16/234 (6.8%)	0.405
Vomiting	89	0/3 (0%)	5/150 (3.3%)	1
Abdominal pain	1	0/7 (0%)	5/234 (2.1%)	1
Abdominal mass	2	0/7 (0%)	2/233 (0.9%)	1
Abdominal effusion	1	0/7 (0%)	1/234 (0.4%)	1
Hepatomegaly	1	0/7 (0%)	1/234 (0.4%)	1
Splenomegaly	1	0/7 (0%)	1/234 (0.4%)	1
Decreased kidney size	1	0/7 (0%)	1/234 (0.4%)	1
Increased kidney size		1/7 (14.3%)	3/234 (1.3%)	
Non-smooth kidney surface	1	1/7 (14.3%)	2/234 (0.9%)	0.085*
Polyuria-polydipsia	105	0/1 (0%)	3/136 (2.2%)	1
Dysuria	98	0/1 (0%)	3/143 (2.1%)	1

Pyometra or penile swelling	1	0/7 (0%)	1/234 (0.4%)	1
Skin lesions and/or pruritus	5	0/7 (0%)	53/230 (23%)	0.354
Neurological signs	1	0/7 (0%)	0/234 (3.8%)	1
Musculoskeletal signs	1	0/7 (0%)	7/234 (3%)	1
At least one clinical sign	3	6/7 (85.7%)	159/232 (68.5%)	0.441
At least one clinical sign compatible with bartonellosis	3	1/7 (14.3%)	67/232 (28.9%)	0.676
<i>Bartonella henselae</i> IgG antibodies (IFAT)	18	3/7 (42.9%)	78/217 (35.9%)	0.705
<i>Mycoplasma</i> spp. infection (PCR)	0	3/7 (42.9%)	43/235 (18.3%)	0.128*
<i>Toxoplasma gondii</i> IgG antibodies (IFAT)	17	3/7 (42.9%)	50/218 (22.9%)	0.360
Feline leukemia virus infection	63	1/7 (14.3%)	6/172 (3.5%)	0.247*
Feline immunodeficiency virus infection	63	1/7 (14.3%)	19/172 (11%)	0.57
Feline Coronavirus IgG antibodies (IFAT)	19	1/7 (14.3%)	32/216 (14.8%)	1

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PCR: polymerase chain reaction; IFAT: immunofluorescence antibody test



**Supplementary data-Table S7.** Univariable associations between the infection status of 242 cats tested for *Bartonella* spp. by PCR and the results of haematology and biochemistry. Variables that were used in the logistic regression model (P value <0.25) are highlighted with an asterisk

			<i>Bartonella</i> spp. PCR status		
Variable	Categories	Missing data	Positive (%)	Negative (%)	P value
HCT	Decreased	0	5/7 (71.4%)	116/235 (49.4%)	0.446
White blood cells	Decreased		0/7 (0%)	16/235 (6.8%)	0.816
White blood cells	Increased	0	2/7 (28.6%)	94/235 (40%)	
Neutrophils	Decreased		2/7 (28.6%)	20/235 (8.5%)	0.127*
Neutrophils	Increased	0	2/7 (28.6%)	52/235 (22.1%)	
Lymphocytes	Decreased		0/7 (0%)	35/235 (14.9%)	0.518
Lymphocytes	Increased	0	1/7 (14.3%)	23/235 (9.8%)	
Monocytes	Increased	0	0/7 (0%)	14/235 (6%)	1
Eosinophils	Decreased		1/7 (14.3%)	6/235 (2.6%)	0.007*
Eosinophils	Increased	0	5/7 (71.4%)	69/235 (29.4%)	
Platelets	Decreased		1/7 (14.3%)	48/235 (20.4%)	0.613
Platelets	Increased	0	1/7 (14.3%)	16/235 (6.8%)	
Total proteins	Decreased	14	1/7 (14%)	31/221 (14%)	0.386
Total proteins	Increased		1/7 (14.3%)	12/221 (5.4%)	
Albumins	Decreased	11	7/7 (100%)	188/224 (83.9)	0.6
Globulins	Decreased	14	0/7 (0%)	10/221 (4.5%)	0.782
Globulins	Increased		3/7 (42.9%)	74/221 (33.5%)	
Urea nitrogen	Decreased	17	1/7 (14.3%)	35/218 (16.1%)	0.641
Urea nitrogen	Increased		1/7 (14.3%)	17/218 (7.8%)	
Creatinine	Increased	12	1/7 (14.3%)	6/223 (2.7%)	0.197*
Glucose	Decreased	15	1/7 (14.3%)	14/220 (6.4%)	0.28
Glucose	Increased		2/7 (28.6%)	109/220 (49.5%)	
Cholesterol	Decreased	16	0/7 (0%)	21/219 (9.6%)	1
Cholesterol	Increased		0/7 (0%)	2/219 (0.9%)	
Triglycerides	Decreased	14	0/7 (0%)	4/221 (1.8%)	0.403

Triglycerides	Increased		1/7 (14.3%)	11/221 (5%)	
Total bilirubin	Increased	11	1/7 (14.3%)	8/224 (3.6%)	0.246*
ALP	Increased	11	2/7 (28.6%)	22/224 (9.8%)	0.157*
ALT	Increased	12	0/7 (0%)	4/223 (1.8%)	1
AST	Increased	11	1/7 (14.3%)	29/224 (12.9%)	1
GGT	Increased	15	0/7 (0%)	4/220 (1.8%)	1
Calcium	Increased	11	1/7 (14.3%)	2/224 (0.9%)	0.089*
Inorganic phosphorus	Increased	15	4/7 (57.1%)	111/220 (50.5%)	1
Potassium	Increased	13	1/7 (14.3%)	66/222 (29.7%)	0.677
Sodium	Decreased	12	1/7 (14.3%)	30/223 (13.5%)	1
Sodium	Increased		0/7 (0%)	18/223 (8.1%)	

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PCR: polymerase chain reaction; HCT: haematocrit; ALP: alkaline phosphatase;  
 ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-  
 glutamyl transpeptidase

**Supplementary data-Table S8.** Univariable associations between the infection status of 242 cats tested for haemoplasma species by PCR and the signalment and historical data that were collected using a standardized questionnaire. Variables that were used in the logistic regression model (P value <0.25) are highlighted with an asterisk

Variables	Categories	Missing data	Haemoplasma species PCR status		P value
			Positive (%)	Negative (%)	
Sex	Male	0	25/46 (54.3%)	105/196 (53.6%)	0.924
	Female		21/46 (45.7%)	91/196 (46.4%)	
Neutered		3	11/45 (24.4%)	60/194 (30.9%)	0.391
Breed	Purebred	6	0/44 (0%)	44/44 (100%)	1
	Crossbreed		2/190 (1%)	190/192 (99%)	
Age (years)		9	2 (0.13-8)	1 (0.13-15)	0.705
Body weight (kg)		19	3 (0.5-7.2)	3 (0.5-8)	0.992
Cat acquisition	Client-owned	58	9/32 (28.1%)	47/152 (30.9%)	0.879
	Stray		20/32 (62.5%)	92/152 (60.5%)	
	Cattery		3/32 (9.4%)	11/152 (7.2%)	
	Pet shop		0/32 (0%)	2/152 (1.3%)	
Current ownership	Client-owned	2	22/45 (48.9%)	115/195 (59%)	0.453
	Stray		20/45 (44.4%)	71/195 (36.4%)	
	Cattery		3/45 (6.7%)	9/195 (4.6%)	
Living conditions	Indoors	7	2/44 (4.5%)	28/191 (14.7%)	0.07*
	Outdoors		42/44 (95.5%)	163/191 (85.3%)	
Living area	Urban	8	31/44 (70.5%)	145/190 (76.3%)	0.417
	Rural		13/44 (29.5%)	45/190 (23.7%)	
Contact with other cats		11	43/44 (97.7%)	174/187 (93%)	0.479
Number of in-contact cats	1-2 cats	56	1/31 (3.2%)	18/155 (11.6%)	0.189*
	3 cats		3/31 (9.7%)	6/155 (3.9%)	
	> 3 cats		26/31 (83.9%)	117/155 (75.5%)	
History of cat-bite wounds		120	4/19 (21.1%)	24/103 (23.3%)	1
Vaccinated against CHPR		69	7/32 (21.9%)	56/141 (39.7%)	0.058*

Use of	79	8/29 (27.6%)	75/134 (56%)	0.006*
ectoparasiticides				
Flea infestation	6	14/45 (31.1%)	49/191 (25.7%)	0.457
Tick infestation	7	1/45 (2.2%)	4/190 (2.1%)	1

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PCR: polymerase chain reaction; CHPR: calicivirus, herpesvirus-1, panleukopenia, rabies

**Supplementary data-Table S9.** Univariable associations between the infection status of 242 cats tested for haemoplasma species by PCR and the presence of clinical signs, the results of serology for *Bartonella henselae* and of PCR for *Bartonella* spp. and the results of serology for *Toxoplasma gondii*, feline coronavirus, feline leukemia virus and feline immunodeficiency virus. Variables that were used in the logistic regression model ( $P$  value  $<0.25$ ) are highlighted with an asterisk

Clinical sign	Missing data	Haemoplasma species PCR status		$P$ value
		Positive (%)	Negative (%)	
Weight loss	76	7/26 (26.9%)	25/140 (17.9%)	0.282
Anorexia (previous 3 weeks)	66	8/27 (29.6%)	24/149 (16.1%)	0.107*
Depression-lethargy	1	6/46 (13%)	24/195 (12.3%)	0.892
Weakness	1	2/46 (4.3%)	4/195 (2.1%)	0.322
Dehydration	2	4/46 (8.7%)	14/194 (7.2%)	0.756
Fever	5	3/46 (6.5%)	3/191 (1.6%)	0.089*
Lymphadenomegaly	3	3/45 (6.7%)	8/194 (4.1%)	0.438
Abnormal lung sounds	3	2/46 (4.3%)	9/193 (4.7%)	1
Cough	0	3/46 (6.5%)	5/196 (2.6%)	0.179*
Dyspnea	2	1/46 (2.2%)	2/194 (1%)	0.473
Signs of rhinitis	2	10/46 (21.7%)	21/194 (10.8%)	0.047*
Ocular signs	5	13/46 (28.3%)	37/191 (19.4%)	0.185*
Gingivostomatitis	1	8/46 (17.4%)	30/195 (15.4%)	0.737
Glossitis	1	3/46 (6.5%)	14/195 (7.2%)	1
Vomiting	89	1/25 (4%)	4/128 (3.1%)	1
Diarrhea	94	1/23 (4.3%)	5/125 (4%)	1
Constipation	98	0/22 (0%)	2/122 (1.6%)	1
Abdominal pain	1	1/46 (2.2%)	4/195 (2.1%)	1
Abdominal mass	2	0/46 (0%)	2/194 (1%)	1
Abdominal effusion	1	0/46 (0%)	1/195 (0.5%)	1
Hepatomegaly	1	0/46 (0%)	1/195 (0.5%)	1
Splenomegaly	1	0/46 (0%)	1/195 (0.5%)	1
Decreased kidney size	1	0/46 (0%)	1/195 (0.5%)	0.657
Increased kidney size		1/46 (2.2%)	3/195 (1.5%)	
Non-smooth kidney surface	1	1/46 (2.2%)	2/195 (1%)	0.472

Polyuria-polydipsia	105	0/19 (0%)	3/118 (2.5%)	1
Dysuria	98	0/21 (0%)	3/123 (2.4%)	1
Pyometra or penile swelling	1	0/46 (0%)	1/195 (0.5%)	1
Skin lesions and/or pruritus	5	11/46 (23.9%)	42/191 (22%)	0.779
Musculoskeletal signs	1	1/46 (2.2%)	6/195 (3.1%)	1
At least one clinical sign	3	28/46 (60.9%)	137/193 (71%)	0.182*
At least one clinical sign compatible with haemoplasmosis	3	9/46 (19.6%)	37/193 (19.2%)	0.951
<i>Bartonella</i> spp. infection (PCR)	0	3/46 (6.5%)	4/196 (2%)	0.128*
<i>Bartonella henselae</i> IgG antibodies (IFAT)	18	18/44 (40.9%)	63/180 (35%)	0.465
<i>Toxoplasma gondii</i> IgG antibodies (IFAT)	17	17/45 (37.8%)	36/180 (20%)	0.012*
Feline leukemia virus infection	63	0/32 (0%)	7/147 (4.8%)	0.355
Feline immunodeficiency virus infection	63	6/32 (18.8%)	14/147 (9.5%)	0.210*
Feline Coronavirus IgG antibodies (IFAT)	19	8/45 (17.8%)	25/178 (14%)	0.529

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PCR: polymerase chain reaction; IFAT: immunofluorescence antibody test

**Supplementary data-Table S10.** Univariable associations between the infection status of 242 cats tested for haemoplasma species by PCR and the results of haematology and biochemistry. Variables that were used in the logistic regression model (P value <0.25) are highlighted with an asterisk

Variable	Categories	Missing data	Haemoplasma species PCR-positive status		
			Positive (%)	Negative (%)	P value
HCT	Decreased	0	27/46 (58.7%)	94/196 (48%)	0.19*
White blood cells	Decreased		1/46 (2.2%)	15/196 (7.7%)	0.166*
White blood cells	Increased	0	23/46 (50%)	73/196 (37.2%)	
Neutrophils	Decreased		4/46 (8.7%)	18/196 (9.2%)	0.336
Neutrophils	Increased	0	14/46 (30.4%)	40/196 (20.4%)	
Lymphocytes	Decreased		6/46 (13%)	29/196 (14.8%)	0.719
Lymphocytes	Increased	0	6/46 (13%)	18/196 (9.2%)	
Monocytes	Increased	0	4/46 (8.7%)	10/196 (5.1%)	0.312
Eosinophils	Decreased		2/46 (4.3%)	5/196 (2.6%)	0.742
Eosinophils	Increased	0	15/46 (32.6%)	59/196 (30.1%)	
Platelets	Decreased		16/46 (34.8%)	33/196 (16.8%)	0.024*
Platelets	Increased	0	3/46 (6.5%)	14/196 (7.1%)	
Total proteins	Decreased	14	7/45 (15.6%)	25/183 (13.7%)	0.444
Total proteins	Increased		4/45 (8.9%)	9/183 (4.9%)	
Albumins	Decreased	11	41/45 (91.1%)	154/186 (82.8%)	0.168*
Globulins	Decreased	14	1/45 (2.2%)	9/183 (4.9%)	0.418
Globulins	Increased		19/45 (42.2%)	58/183 (31.7%)	
Urea nitrogen	Decreased	17	8/45 (17.8%)	28/180 (15.6%)	0.802
Urea nitrogen	Increased		4/45 (8.9%)	14/180 (7.8%)	
Creatinine	Decreased	12	9/45 (20%)	33/185 (17.8%)	0.936
Creatinine	Increased		1/45 (2.2%)	6/185 (3.2%)	
Glucose	Decreased	15	3/45 (6.7%)	12/182 (6.6%)	0.931
Glucose	Increased		21/45 (46.7%)	90/182 (49.5%)	
Cholesterol	Decreased	16	2/45 (4.4%)	19/181 (10.5%)	0.528
Cholesterol	Increased		0/45 (0%)	2/181 (1.1%)	
Triglycerides	Decreased	14	1/45 (2.2%)	3/183 (1.6%)	0.561
Triglycerides	Increased		1/45 (2.2%)	11/183 (6%)	

Total bilirubin	Increased	11	2/45 (4.4%)	7/186 (3.8%)	0.689
ALP	Increased	11	3/45 (6.7%)	21/186 (11.3%)	0.585
ALT	Increased	12	1/45 (2.2%)	3/185 (1.6%)	0.584
AST	Increased	11	5/45 (11.1%)	25/186 (13.4%)	0.677
GGT	Increased	15	1/45 (2.2%)	3/182 (1.6%)	1
Calcium	Decreased	11	1/45 (2.2%)	9/186 (4.8%)	0.839
Calcium	Increased		0/45 (0%)	3/186 (1.6%)	
Inorganic phosphorus	Increased	15	22/45 (48.9%)	93/182 (51.1%)	0.791
Potassium	Increased	13	11/45 (24.4%)	56/184 (30.4%)	0.429
Sodium	Decreased	12	7/45 (15.6%)	24/185 (13%)	0.717
Sodium	Increased		2/45 (4.4%)	16/185 (8.6%)	
Chloride	Decreased	12	17/45 (37.8%)	70/185 (37.8%)	1
Chloride	Increased		0/45 (0%)	2/185 (1.1%)	

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PCR: polymerase chain reaction; HCT: haematocrit; ALP: alkaline phosphatase;  
 ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase



## **5. Article No 4**

**Seroprevalence and risk factors for *Toxoplasma gondii* infection in cats from Greece. Under review**

## **Short Communication**

### **Seroprevalence and risk factors for *Toxoplasma gondii* infection in cats from Greece**

## Abstract

Toxoplasmosis is one of the most important protozoan diseases with a global impact on the health of domestic cats and with zoonotic significance. The aims of this study were to determine the prevalence of seropositivity for *Toxoplasma gondii* in different populations of cats in Greece and to assess risk factors for seropositivity.

A total of 457 cats were prospectively enrolled and a commercially available IFAT kit was used for the detection of anti-*T. gondii* IgG in serum.

Overall, 95 (20.8%) of the 457 cats were seropositive for *T. gondii*. Based on multivariate analysis, factors associated with seropositivity included older age, a history of cat-fight trauma, and lack of vaccination against calicivirus, herpesvirus-1, panleukopenia, and rabies.

This study shows a high prevalence of seropositivity for *T. gondii* in cats in Greece. This implies that toxoplasmosis is still a major public health concern and that optimal strategies for the prevention of infection with *T. gondii* in cats should be established.

**Keywords:** feline, prevalence, protozoan, public health, seropositivity

## Introduction

Toxoplasmosis is one of the most important zoonotic protozoan diseases, with a global impact on the health of domestic cats (Tenter et al., 2000). The causative agent is *Toxoplasma gondii* which infects virtually all warm-blooded animals (intermediate hosts). Domestic cats and other felids are the definitive hosts that excrete oocysts (Su et al., 2003).

The main modes of transmission, to both intermediate and definitive hosts, are congenital infection through the placenta, ingestion of oocyst-contaminated food or water and ingestion of raw infected tissues, with the latter being the primary source of infection in cats (Hill and Dubey, 2002). Other, less important, modes of transmission include blood transfusion, lactation and organ transplantation (Bernsteen et al., 1999; Powell et al., 2001).

Reported risk factors for *T. gondii* seropositivity in cats include male gender, older age, being non-purebred, and hunting and eating intermediate hosts (rodents and birds) or mechanical vectors such as cockroaches and earthworms (Gauss et al., 2003; Maruyama et al., 2003; Voltaire et al., 2005; López et al., 2011).

The global prevalence of seropositivity against *T. gondii*, based on meta-analyses, has been reported to be 35% and 32.9% in cats and in humans, respectively (Bigna et al., 2020; Montazeri et al., 2020). Because of the vital role of cats for human infection, the high seropositivity rate highlights the need for current and comprehensive data on the epidemiology of *T. gondii* infection in cats. Only limited seroepidemiological data are available on *T. gondii* infection of cats in Greece (Chatzis et al., 2014), and there is no study investigating the risk factors for seropositivity. The objectives of the present study were to determine the prevalence of seropositivity against *T. gondii* in different populations of cats living in different areas of Greece and to assess risk factors related to signalment and history.

## Materials and methods

### *Ethics approval*

The study protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Thessaly (13/16-6-15). Handling of these animals was in compliance with the European Communities Council Directive 2010/63/EU and state laws.

### *Study population*

Cats from four different geographic areas of Greece (Attica, Thessaly, Crete, and Macedonia) were prospectively enrolled, between November 2013 and November 2016, as part of a previous study (Kokkinaki et al., 2021), and they were divided into three groups: client-owned cats, stray cats, and cats living in breeding catteries. These cats were presented for wellness examination, vaccination, neutering and/or medical treatment. Stray cats often originated from cat-dense environments (i.e., rescue colonies) and their age was estimated based on body size, dentition and other physical characteristics (Hale, 2005; Bellows et al., 2016; DiGangi et al., 2020).

Inclusion criteria included: a) body weight of  $\geq 0.5$  kg, and b) an informed consent form signed by the owner or the rescuer. The cats were enrolled randomly, and no preference was given to their health status. Signalment and historical data were collected using a standardized questionnaire.

### *Sample collection and laboratory analyses*

A total of 5 mL of blood was collected by jugular venipuncture from each cat. Serum was harvested following centrifugation at 3000 rpm for 20 min and stored at  $-80^{\circ}\text{C}$  until indirect fluorescent antibody test (IFAT) for anti-*T. gondii* IgG was performed.

Anti-*T. gondii* IgG antibodies were detected using a commercially available IFAT kit (Biopronix Product Line, Agrolabo S.p.a., Italy) according to the manufacturer's instructions. The cut-off value was 1/50, and all slides were examined by fluorescence microscopy (Olympus, Japan) at 400x magnification.

### *Statistical analysis*

For univariate analysis, categorical data, regarding signalment and historical information, were compared between *T. gondii* seropositive and seronegative cats using Pearson's  $\chi^2$  or Fischer's exact tests. The normality of the distribution of the continuous variables was tested using the Kolmogorov-Smirnov test. Normally distributed data are presented as means  $\pm$  standard deviation and were compared between seropositive cats and seronegative cats using independent sample t-tests. Not normally distributed data are presented as medians and ranges and were compared between seropositive cats and seronegative cats using Mann Whitney U tests.

Variables that, in the univariate analysis, were different at 25% level of significance, between seropositive and seronegative cats were selected as candidates for an initial logistic regression model. The initial model was subsequently reduced in a stepwise manner until only significantly different ( $P < 0.05$ ) variables remained. Odds ratios (OR) derived from the reduced model were interpreted as measures of the risk of seropositivity.

The analyses were performed using Stata 13 (Stata Corp, College Station, TX) and SPSS 23 for Windows (IBM Corp, Armonk, NY).

## Results

A total of 457 cats were prospectively enrolled in the study. The age of these cats ranged from 6 weeks to 17 years (median: 2 years). Two hundred and forty-two cats (53%) were males (86 neutered; 35.5% of the male cats), and 215 (47%) were females (52 neutered; 24.2% of the female cats). Fifteen cats (3.3%) were purebred, 427 (93.4%) were crossbred, and for 15 (3.3%) cats the breed was not recorded. Ninety-nine cats (21.7%) lived exclusively indoors, 336 (73.5%) outdoors, and for 22 cats (4.8%) living status was not recorded. Two hundred and fifty-eight cats (56.5%) were living in Attica, 73 (16%) in Thessaly, 79 (17.3%) in Crete, and 47 (10.3%) in Macedonia. Two hundred and sixty-nine cats (58.9%) were client-owned, 158 (34.6%) were stray, 21 (4.6%) were living in catteries, and for nine cats (2%) current ownership was not recorded. Three hundred and sixty-six cats (80.1%) lived in urban areas, 68 (14.9%) in rural areas, while for 23 (5%) cats their habitat was not recorded.

Of the 457 cats, a total of 95 (20.8%) were seropositive for *T. gondii*. The univariate associations between seropositivity and signalment or historical data are presented in Table 1.

Multivariate analysis indicated three factors that were independently associated with *T. gondii*-seropositivity (Table 2). *Toxoplasma gondii*-seropositive cats were older, were more likely to have history of cat-fight trauma, and more likely to be unvaccinated against calicivirus, herpesvirus-1, panleukopenia and rabies (CHPR), compared to seronegative cats.

## Discussion

This is one of the most extensive prospective studies on natural infection with *T. gondii* in cats, and the first study reporting risk factors for seropositivity in Greece.

Overall, 20.8% of the cats were seropositive for *T. gondii*. In addition, older age, history of cat-fight trauma and non-vaccination against calicivirus, herpesvirus-1, panleukopenia and rabies were positively associated with *T. gondii*-seropositivity.

The seroprevalence identified here is similar to that from a previous prospective study in Greece, where it was reported to be 21% (Chatzis et al., 2014). In addition, the seroprevalence in our study is similar to that reported in other countries, including Italy, Portugal, United Kingdom, the Netherlands, Argentina, Brazil and China (Bennett et al., 2011; López et al., 2011; Opsteegh et al., 2012; Spada et al., 2013; Esteves et al., 2014; Liu et al., 2014; Pereira et al., 2018).

Globally, studies evaluating the seroprevalence for *T. gondii* have shown a wide variation. Seroprevalence has been reported to be 41-60.8% in Northern Europe (Must et al., 2015; Sævik et al., 2015), 10-84.7% in Southern Europe (Millan et al., 2009; Miro et al., 2011), 19.2-65.5% in Western Europe (Bennett et al., 2011; Afonso et al., 2013), 14.7-39.3% in Eastern Europe (Pavlova et al., 2016; Shuralev et al., 2018), 47-81.3% in Central Europe (Sroka and Szymańska, 2012; Shuralev et al., 2018), 1.1-100% in North America (Bevins et al., 2012; Jimenez-Coello et al., 2013), 0-82.8% in South America, 25% in Central America (Furtado et al., 2015; Teixeira et al., 2016; Rengifo-Herrera et al., 2017), 50-97.5% in North Africa, 4.4-36.2% in West Africa, 3.9% in South Africa (Al-Kappany et al., 2010; Kamani et al., 2010; Ayinmode et al., 2017; Lopes et al., 2017; Yekkour et al., 2017), and 2.2-82.8% in Asia (Hong et al., 2013; Asgari et al., 2018). This tremendous variability may reflect differences in the demographics and the geographic origin of the cats, the accuracy of the diagnostic tests and/or the overtime changes in the prevalence of these infections.

In our study, *T. gondii*-seropositive cats were significantly older than *T. gondii*-seronegative cats. This finding is consistent with previous studies, and may be explained by the fact that older cats have more time to be exposed to the parasite (Gauss et al., 2003; Maruyama et al., 2003; Voltaire et al., 2005).

Our study showed that cats with a history of cat-bite wounds were more likely to be *T. gondii*-seropositive than cats with no such history. To our knowledge, this is the first time that a significant association between cat-bite wounds and seropositivity to *T. gondii* is reported. The major route of transmission among cats is the ingestion of bradyzoites within tissue cysts. However, transmission through bite wounds that introduce saliva containing tachyzoites may also occur (Frenkel, 1973). In this case, the biting cat must be in the acute phase of infection, in order to have tachyzoites in

blood (parasitemia) and body secretions (Terragna et al., 1984; Burney et al., 1999; Hill and Dubey, 2002). However, cats with history of cat-bite wounds are more likely to live outside and thus to hunt and eat intermediate hosts, which is a well-established risk factor for *T. gondii*-seropositivity. Therefore, we suggest that the association between cat-bite wounds and seropositivity most likely reflects the consumption of the intermediate hosts by the cats.

Lack of vaccination against CHPR was found to be an independent risk factor for *T. gondii*-seropositivity. This is consistent with the results of a recent study on the epidemiology of *T. gondii* in 155 cats from Cyprus (Attipa et al., 2021) and presumably, reflects the better quality of life and veterinary care of the vaccinated cats compared to unvaccinated ones. Unvaccinated cats have an increased chance to become infected by calicivirus, herpesvirus-1 and/or panleukopenia virus, and to develop clinical illness. Concomitant illnesses render cats more susceptible to *T. gondii* infection. Also, they may lead to reactivation of tissue cysts in chronically infected but seronegative cats, with subsequent release of bradyzoites and seroconversion (Dubey and Lappin, 2012). Finally, unvaccinated cats are more likely to be stray and thus to be more prone to eat intermediate hosts.

## Conclusions

This study shows a high prevalence of seropositivity for *T. gondii* among cats in Greece. Thus, feline toxoplasmosis should be considered a major public health concern in this country, necessitating the establishment of optimal prevention strategies, taking into consideration the high-risk groups of cats identified in this study.

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**Table 1.** Univariable associations between seropositivity for *Toxoplasma gondii* among 457 cats and the signalment and historical data that were collected using a standardized questionnaire. Variables that were used in the logistic regression model ( $P$  value  $<0.25$ ) are highlighted with an asterisk

Variables	Categories	Missing data	Anti- <i>T. gondii</i> IgG antibodies		
			Positive (%)	Negative (%)	$P$ value
Sex	Male	0	39/95 (41.1%)	203/362 (56.1%)	0.009*
	Female		56/95 (58.9%)	159/362 (43.9%)	
Neutering		6	26/93 (28%)	112/358 (31.3%)	0.535
Breed	Purebred	15	1/91 (1.1%)	14/351 (4%)	0.326
	Crossbreed		90/91 (98.9%)	337/351 (96%)	
Age (years)		23	2.5 (0.13-17)	1.05 (0.13-15)	$<0.001^*$
Body weight (kg)		46	3.5 (0.85-7.2)	3.2 (0.5-9)	0.173*
Cat acquisition	Client-owned	95	14/75 (18.7%)	61/287 (21.3%)	0.625
	Stray		58/75 (77.3%)	202/287 (70.4%)	
	Cattery		3/75 (4%)	21/287 (7.3%)	
	Pet shop		0/75 (0%)	3/287 (1%)	
Current ownership	Client-owned	9	51/93 (54.8%)	218/355 (61.4%)	0.433
	Stray		38/93 (40.9%)	120/355 (33.8%)	
	Cattery		4/93 (4.3%)	17/355 (4.8%)	
Living conditions	Indoors	22	9/90 (10%)	90/345 (26.1%)	0.001*
	Outdoors		81/90 (90%)	255/345 (73.9%)	
Living area	Urban	23	72/90 (80%)	294/344 (85.5%)	0.204*
	Rural		18/90 (20%)	50/344 (14.5%)	
Contact with other cats		57	72/80 (90%)	272/320 (85%)	0.249*
Number of in-contact cats	0 cats	131	8/56 (14.3%)	48/270 (17.8%)	0.669
	1-2 cats		9/56 (16.1%)	48/270 (17.8%)	
	3 cats		3/56 (5.4%)	8/270 (3%)	
	>3 cats		36/56 (64.3%)	166/270 (61.5%)	
History of cat-bite wounds		256	12/29 (41.4%)	35/172 (20.3%)	0.013*
Vaccinated against CHPR		119	25/72 (34.7%)	138/266 (51.9%)	0.01*

Use of ectoparasiticide	190	27/53 (50.9%)	132/214 (61.7%)	0.154 <sup>*</sup>
Flea infestation	11	27/92 (29.3%)	63/354 (17.8%)	0.014 <sup>*</sup>
Tick infestation	12	1/92 (1.1%)	6/353 (1.7%)	1
Consumption of prey, raw meat, and/or unpasteurized milk	245	24/35 (68.6%)	70/177 (39.5%)	0.002 <sup>*</sup>

IFAT: immunofluorescence antibody test; CHPR: calicivirus, herpesvirus-1,  
panleukopenia, rabies

**Table 2.** Multivariate analysis of risk factors for *Toxoplasma gondii* seropositivity

<b>Variables</b>	<b>Odds Ratio</b>	<b>Confidence Interval</b>	<b>P value</b>
Age (years)	1.33	1.15 - 1.54	< 0.001
History of cat-bite wounds	3.88	1.54 - 9.81	0.004
Vaccinated against CHPR	0.1	0.07 - 0.55	0.002

CHPR: calicivirus, herpesvirus-1, panleukopenia, rabies



## **6. Article No 5**

**Prevalence of feline hyperthyroidism in Greece.** Under review

## **Short Communication**

### **Prevalence of feline hyperthyroidism in Greece**

## **Abstract**

Hyperthyroidism is the most common endocrine disease of cats and usually occurs in middle-aged to older animals. The primary aim of this study was to determine the prevalence of feline hyperthyroidism in Greece, and the secondary aim was to compare the clinical signs, haematological and serum biochemical findings between hyperthyroid and non-hyperthyroid cats.

A total of 80 cats, six years or older, were prospectively enrolled. The diagnosis of hyperthyroidism was based on serum concentration of total thyroxine and of free thyroxin (both measured by solid-phase chemiluminescent competitive immunoassays).

Hyperthyroidism was diagnosed in 4/80 (5%) of the cats. Clinical and laboratory findings that were significantly more common in these hyperthyroid cats compared to the non-hyperthyroid cats included a palpable thyroid nodule, depression-lethargy, dyspnoea and decreased blood urea nitrogen concentration.

Feline hyperthyroidism is quite common in Greece and routine measurement of thyroid hormone concentrations in serum should be considered in all middle-aged and older cats.

*Keywords:* biochemistry; clinical signs; haematology; hormone; epidemiology; thyroid

## Introduction

Hyperthyroidism is a multi-systemic disorder resulting from excessive circulating concentrations of thyroid hormones, namely thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>). It occurs mainly in middle-aged and older cats, with a reported age range of 2-25 years and a median age at the time of diagnosis of 11.5-15.1 years (Milner et al., 2006; Williams et al., 2010; Peterson, 2013a; Peterson and Broome, 2015; Puig et al., 2015; Grossi et al., 2019). Benign adenoma or adenomatous hyperplasia of the thyroid gland are the most common causes of the disease, accounting for 97-99% of the cases (Turrel et al., 1988; Peterson et al., 1994; Naan et al., 2006).

A wide range of clinical signs is seen in hyperthyroid cats, including weight loss, increased appetite, polyuria/polydipsia, vomiting, diarrhoea, hyperactivity, tachypnoea, tachycardia, and palpable goitre (Broussard et al., 1995; Frenais et al., 2009; Nussbaum et al., 2015; Grossi et al., 2019). Less commonly, cats present with the apathetic form of the disease, that is characterized by lethargy, weakness and decreased appetite, which is usually accompanied by concurrent severe systemic diseases (Thoday and Mooney, 1992; Bucknell, 2000; Frenais et al., 2009; Nussbaum et al., 2015; Grossi et al., 2019).

The most common haematologic and serum biochemistry abnormalities associated with feline hyperthyroidism include anaemia, leucocytosis, increased activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), decreased calcium and increased inorganic phosphorous concentrations and decreased potassium osmolalities (Peterson and Ferguson, 1983; Thoday and Mooney, 1992; Broussard et al., 1995; Milner et al., 2006; Naan et al., 2006; Frenais et al., 2009; Williams et al., 2013; Hiebert et al., 2020).

Hyperthyroidism is the most common endocrinopathy of cats, with 1-4% prevalence in the general feline population and at least 6% in cats older than 9 years of age (Milner et al., 2006; Trepanier, 2006; Wakeling et al., 2011). Since its initial description (Holzworth et al., 1980), the prevalence (or the frequency of diagnosis) of the disease has been steadily increasing worldwide (McLean et al., 2017), and there is strong evidence that its prevalence differs among geographical locations (Scarlett et al., 1988). Depending on the selection criteria (mainly the age) of the cats enrolled in each study, reported prevalence rates vary widely, from 0.2% to 21.1% in Europe, from 0.1 to 2% in North America, and from 3.9% to 8.9% in Asia (Edinboro et al.,

2004; De Wet et al., 2009; Wakeling et al., 2011; McLean et al., 2017). Until now, the prevalence of feline hyperthyroidism in Greece remains unknown.

The primary aim of this study was to determine the prevalence of feline hyperthyroidism in Greece. The secondary aim was to compare the clinical signs as well as the haematological and serum biochemical findings between hyperthyroid and non-hyperthyroid cats.

## **Materials and Methods**

### *Ethics approval*

The study protocol was reviewed and approved by the Animal Ethics Committee of the Faculty of Veterinary Science, University of Thessaly (licence No: 13/16-6-15). Handling of the cats was in compliance with the European Communities Council Directive 2010/63/EU and State laws.

### *Study population*

Cats from four different areas of Greece (i.e. Attica, Thessaly, Crete, and Macedonia) were prospectively enrolled, by three clinicians (KCGK, PGX, MEM), between March 2014 and July 2016. Client-owned cats, cats living in catteries, and stray cats that were presented by their owners or by volunteer cat-rescue groups for wellness examination, vaccination, neutering and/or medical treatment, were enrolled if they were at least 6 years old [the age of stray cats was estimated based on body size, dentition and other physical characteristics (Hale, 2005; Bellows et al., 2016; DiGangi et al., 2020)] and an informed consent form was signed by the owner or the rescuer. The only exclusion criterion was previous diagnosis and/or treatment of hyperthyroidism.

Signalment and historical data were collected using a standardized questionnaire followed by a thorough physical examination and blood sampling.

### *Sample collection and laboratory analyses*

A total of 5 ml of blood was collected from the jugular vein. One ml was immediately transferred into a EDTA-anticoagulated tube and was used for haematology, whereas the remaining blood was centrifuged, and serum was separated and stored at -80 °C until further use for hormone assays and biochemistry analysis.

Serum total T<sub>4</sub> (tT<sub>4</sub>) concentration was measured by a solid-phase chemiluminescent competitive assay (Immulite 2000 Canine Total T<sub>4</sub>, Siemens Healthcare Diagnostics, Deerfield, IL) with a reference interval of 0.78-3.82 µg/dl. Serum free T<sub>4</sub> (fT<sub>4</sub>) concentration was measured by a solid-phase chemiluminescent competitive immunoassay (Immulite 2000 Veterinary Free T<sub>4</sub>, Siemens Healthcare Diagnostics, Llanberis, Gwynedd, UK) with a reference interval of 0.7-2.6 ng/dl. A cat was considered hyperthyroid if: a) tT<sub>4</sub> concentration was increased or b) tT<sub>4</sub> concentration was in the upper half of the reference interval (i.e. >2.3 µg/dl), fT<sub>4</sub> concentration was increased and there was a palpable goitre (Peterson and Broome, 2015).

Complete blood count (CBC) was performed within 12 h of blood collection, with one of three different analysers (Sysmex pocH-100i, ADVIA 2120i Siemens, ADVIA 120 Siemens), depending on sampling area. The results for each haematology parameter were classified as increased, normal, or decreased, based on the reference intervals determined for the specific analyser.

Complete serum biochemistry panels was performed using an automated analyser (Roche/Hitachi MODULAR ANALYTICS D 2400 module, Roche Diagnostics, Switzerland, CH).

### *Statistical analysis*

Categorical data from signalment, history, clinical examination, haematology, and serum biochemistry were compared between hyperthyroid and euthyroid cats by either Pearson's  $\chi^2$  or Fisher's exact test. The distribution of continuous variables was tested by a Kolmogorov-Smirnov test. Normally distributed variables are presented as mean  $\pm$  standard deviation (SD) and were compared between hyperthyroid and euthyroid cats by independent samples *t*-test. Non-normally distributed variables are presented as median and range and were compared between hyperthyroid and euthyroid cats by the Mann Whitney *U* test. Analyses were done using SPSS 23 for Windows (IBM Corp, Armonk, NY) and the level of significance was set at  $P < 0.05$ .

## **Results**

A total of 80 cats were included, 70 (87.5%) of them were sick and 10 (12.5%) were clinically healthy.

A total of 4/80 (5%) cats were diagnosed with hyperthyroidism. All of them were sick, and 3/4 (75%) had increased tT<sub>4</sub> (5.39 µg/dl, 6.32 µg/dl, and 8.49 µg/dl, respectively) and fT<sub>4</sub> (4.73 ng/dl, 5.56 ng/dl and >6 ng/dl, respectively) concentration. In addition, two of these three cats with the increased tT<sub>4</sub> and fT<sub>4</sub> presented the apathetic form of hyperthyroidism, while the third cat presented the classical clinical signs of the disease. The fourth cat had tT<sub>4</sub> concentration in the upper half of the reference range (2.35 µg/dl), increased fT<sub>4</sub> concentration (3.26 ng/dl), palpable goitre and presented the apathetic form of hyperthyroidism. This cat also presented with clinical signs of upper respiratory infection, laboratory investigation revealed anaemia, decreased serum albumin and increased serum globulin concentration, it was seropositive for feline immunodeficiency virus, *Toxoplasma gondii* IgG antibodies and *Bartonella* spp. IgG antibodies and it was blood PCR-positive for *Mycoplasma* spp.

Of the remaining 76/80 (95%) euthyroid cats 66 (86.8%) were sick and 10 (13.2%) were clinically healthy. Their tT<sub>4</sub> (0.63-3.27 µg/dl) and fT<sub>4</sub> (0.4-2.29 ng/dl) concentrations were below the upper limit of the reference interval, respectively.

The age of the four hyperthyroid cats ranged from 6 to 13 years (median: 9 years). Two (50%) of them were neutered males, and 2 (50%) spayed females. All were DSH and their body weight was 2.75 ± 0.35 kg. The age of the 76 euthyroid cats ranged from 6 to 17 years (median: 8 years). Thirty-nine (51.3%) of them were males (29 castrated) and 37 (48.7%) females (26 spayed). Four (5.3%) were purebred, 70 (92.1%) DSH, and the breed of 2 (2.6%) cats was not recorded. Their body weight was 4.35 ± 1.41 kg. There was no significant difference in the age ( $P = 0.975$ ), sex ( $P = 1$ ) or body weight ( $P = 0.115$ ) between hyperthyroid and euthyroid cats ( $P$  value > 0.05).

The frequency of clinical manifestations, based on history and clinical examination (Table 1), and of haematology and serum biochemistry abnormalities (Table 2) were compared between hyperthyroid and euthyroid cats. The frequency of palpable goitre, depression-lethargy and dyspnoea was significantly higher in hyperthyroid compared to euthyroid cats (Table 1). Also, decreased blood urea nitrogen (BUN) concentration was more common in the hyperthyroid cats (Table 2).

## Discussion

This is the first study reporting the prevalence of feline hyperthyroidism in Greece. The prevalence was found to be 5% among cats with an age range of 6 to 17 years. Palpable goitre, depression-lethargy, dyspnoea and decreased BUN concentration were more common in hyperthyroid compared to age-matched euthyroid cats.

The diagnostic sensitivity of  $tT_4$  for feline hyperthyroidism varies from 90% to 95.2% but can be as low as 65% in the early stages of the disease and in cats with concurrent systemic illness (Milner et al., 2006; Peterson, 2013b; Peterson and Broome, 2015; Peterson et al., 2015). However, the diagnostic sensitivity of  $fT_4$  in hyperthyroid cats with  $tT_4$  concentration within the reference interval is approximately 95% (Peterson, 2006). Since all 76 cats that were considered euthyroid had  $tT_4$  and  $fT_4$  concentrations within the reference interval, the possibility of having misclassified some hyperthyroid cats as euthyroid is small, although it cannot be definitively excluded.

The reported diagnostic specificity of  $tT_4$  is very high, typically varying between 98% and 100% (Peterson, 2006, 2013b; Peterson and Broome, 2015). Also, the diagnostic specificity of increased  $fT_4$  in cats with high-normal  $tT_4$  and palpable goitre is high (Peterson, 2013b) and for this reason the single cat with normal  $tT_4$  and an increased serum  $fT_4$  concentration was considered hyperthyroid. The authors acknowledge that it would have been ideal, for this particular cat, to have the measurement of thyroid hormones repeated and/or to perform alternative diagnostic tests (e.g. scintigraphy,  $T_3$  suppression test) to definitively confirm the diagnosis. Unfortunately, this was not feasible due to the design of the study and the long time period between the enrolment of this cat and the measurement of its thyroid hormone concentrations.

Our results are comparable with those of most previous studies, where the prevalence of hyperthyroidism in middle-aged and older cats was found to vary between 2% and 12% (Kraft and Buchler, 1999; Wakeling and Melian, 2005; Sassnau, 2006; Wakeling et al., 2011; Dias Neves and Horspool, 2014; O'Neill et al., 2014; Stephens et al., 2014; Köhler et al., 2016). However, higher figures have also been reported, such as 20% in Poland (Gójska Zygnier et al., 2014) and 21.1% in Ireland (Bree et al., 2018), and the prevalence of the disease is increasing worldwide, thus necessitating routine clinical (neck palpation for goitre) and laboratory



(measurement of tT<sub>4</sub> concentration) testing for hyperthyroidism in all middle-aged and old-aged cats.

The reported diagnostic sensitivity of the palpation of one or two enlarged thyroid nodules varies from 71% to 96% (Paepe et al., 2008; Hibbert et al., 2009), depending on the palpation technique (classical and/or as described Norsworthy) (Norsworthy et al., 2002) the conformation of the neck of the cat, the size of the thyroid nodule (very small nodules may not be palpable and large nodules may descent and enter into the thoracic cavity) and the source of excessive thyroid hormone production (thyroid gland or ectopic thyroid tissue) (Broome, 2006; Peterson, 2013b; Peterson and Broome, 2015). The above may explain why goitre was not palpable in 1/4 hyperthyroid cats in the present study. In contrast to the sensitivity, the diagnostic specificity of this examination is considered poor because a palpable nodule may represent non-functional thyroid tissue (e.g. thyroid cyst, thyroiditis), early stages of functional thyroid tissue that does not produce enough hormones to result in clinical hyperthyroidism, enlarged parathyroid glands, enlarged lymph nodes, and also because of misinterpretation of the finding by an inexperienced examiners (Peterson, 2006; Paepe et al., 2008; Wakeling et al., 2011; Peterson, 2013b). Even when 30 hyperthyroid and euthyroid cats were examined, independently, by three experienced clinicians, that were blinded to the thyroid status of each cat, the specificity of palpation ranged from 63.3% to 66.7% (Paepe et al., 2008). In light of these data, the high specificity (98.7%) of thyroid palpation (Table 1) was a surprising finding of our study. Furthermore, we confirmed previous statements that cats with palpable nodules in the ventral neck region are significantly more likely to be hyperthyroid compared to cats without such palpable nodules (McLean et al., 2017; Bree et al., 2018).

The majority (3/4) of the hyperthyroid cats in our study presented the apathetic form of the disease with depression or lethargy instead of hyperactivity and aggressiveness (Thoday and Mooney, 1992; Bucknell, 2000). Most likely the apathetic form occurred due to comorbidities or due to severe complications of hyperthyroidism, such as heart failure that could also explain the increased prevalence of dyspnoea. The unexpected high prevalence of apathetic hyperthyroidism was probably the result of the inclusion and exclusion criteria of the study: cats with the classical clinical signs of the disease (hyperactivity, aggression, etc.) are more likely

to be tested and diagnosed with hyperthyroidism by their primary care veterinarian and such a previous diagnosis was an exclusion criterion for our study.

In the current study, dyspnoea was more common in hyperthyroid compared to euthyroid cats. The same has been reported in a prospective study of 508 cats from Ireland (Gallagher and Mooney, 2013) and the prevalence of dyspnoea among hyperthyroid cats was reported to range from 11% and 32% (Peterson and Ferguson, 1983; Grossi et al., 2019). Dyspnoea in hyperthyroid cats has been attributed to congestive heart failure, heat intolerance, and/or various respiratory comorbidities (Syme, 2007; Sangster et al., 2013).

Finally, the increased frequency of significantly lower BUN concentration in hyperthyroid than in euthyroid cats, although not previously reported, can be explained by the increased glomerular filtration rate, which is present in cats with hyperthyroidism (Syme, 2007).

Limitations of this study include the relatively small number of cats tested, and the inability to definitively confirm hyperthyroidism in the cat with normal  $tT_4$  and high  $fT_4$  concentration. Future prospective studies, addressing a larger number of cats and using additional diagnostic modalities to confirm the diagnosis in equivocal cases, are warranted to determine the risk factors for the appearance of the disease in Greece and to investigate further its clinical and clinicopathologic manifestations.

## **Conclusion**

The results of this study indicate that hyperthyroidism is relatively common in cats in Greece, from the age of 6 years and above. The disease must be considered in any sick middle-aged and old-aged cat, especially if it presents palpable goitre, depression-lethargy, dyspnoea and/or decreased BUN concentration.

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**Table 1.** Comparison of the frequency of clinical manifestations, based on history and clinical examination, between four hyperthyroid and 76 euthyroid cats (asterisks indicate variables that differ significantly between the two groups of cats)

Clinical sign	Missing data	Hyperthyroid (%)	Euthyroid (%)	<i>P</i> value
Weight loss	9	2/3 (66.7%)	15/68 (22.1%)	0.14
Anorexia (previous 3 weeks)	6	2/4 (50%)	22/70 (31.4%)	0.591
Increased appetite	9	0/4 (0%)	2/67 (3%)	1
Depression-lethargy	0	3/4 (75%)	15/76 (19.7%)	0.034*
Weakness	0	1/4 (25%)	3/76 (3.9%)	0.189
Dehydration	0	2/4 (50%)	15/76 (19.7%)	0.197
Fever	0	0/4 (0%)	4/76 (5.3%)	1
Lymphadenomegaly	0	0/4 (0%)	7/76 (9.2%)	1
Abnormal lung sounds	0	0/4 (0%)	3/76 (3.9%)	1
Cough	0	1/4 (25%)	0/76 (0%)	0.05
Dyspnoea	0	2/4 (50%)	2/76 (2.6%)	0.011*
Ocular signs	0	1/4 (25%)	12/76 (15.8%)	0.515
Gingivostomatitis	0	2/4 (50%)	22/76 (28.9%)	0.579
Vomiting	6	0/3 (0%)	8/71 (11.3%)	1
Diarrhoea	6	0/3 (0%)	4/71 (5.6%)	1
Constipation	8	0/3 (0%)	1/69 (1.4%)	1
Abdominal pain	0	0/4 (0%)	2/76 (2.6%)	1
Abdominal mass	0	0/4 (0%)	2/76 (2.6%)	1
Abdominal effusion	0	0/4 (0%)	2/76 (2.6%)	1
Hepatomegaly	0	0/4 (0%)	1/76 (1.3%)	1
Splenomegaly	0	1/4 (25%)	0/76 (0%)	0.05
Increased kidney size	0	1/4 (25%)	2/76 (2.6%)	0.144

Non-smooth kidney surface	0	0/4 (0%)	2/76 (2.6%)	1
Polyuria-polydipsia	10	0/3 (0%)	6/67 (9%)	1
Dysuria	7	0/3 (0%)	3/70 (4.3%)	1
Skin lesions and/or pruritus	0	2/4 (50%)	24/76 (31.6%)	0.592
Neurological signs	0	0/4 (0%)	2/76 (2.6%)	1
Musculoskeletal signs	0	0/4 (0%)	4/76 (5.3%)	1
Palpable goitre	0	3/4 (75%)	1/76 (1.3%)	<0.001*
At least one clinical sign/symptom	0	4/4 (100%)	66/76 (86.8%)	1
At least one clinical sign/symptom compatible with hyperthyroidism	0	4/4 (100%)	42/76 (55.3%)	0.133



**Table 2.** Comparison of the frequency of haematology and serum biochemistry abnormalities between four hyperthyroid and 76 euthyroid cats (asterisks indicate variables that differ significantly between the two groups of cats)

Variable	Categories	Missing data	Hyperthyroid (%)	Euthyroid (%)	<i>P</i> value
HCT	Decreased	26	1/4 (25%)	16/50 (32%)	1
White blood cells	Decreased	26	0/4 (0%)	7/50 (14%)	1
White blood cells	Increased		1/4 (25%)	11/50 (22%)	
Neutrophils	Decreased	26	1/4 (25%)	6/50 (12%)	0.306
Neutrophils	Increased		1/4 (25%)	7/50 (14%)	
Lymphocytes	Decreased	26	1/4 (25%)	14/50 (28%)	1
Monocytes	Increased	26	0/4 (0%)	1/50 (2%)	1
Eosinophils	Decreased	26	0/4 (0%)	3/50 (6%)	1
Eosinophils	Increased		1/4 (25%)	9/50 (18%)	
Platelets	Decreased	26	1/4 (25%)	13/50 (26%)	1
Platelets	Increased		0/4 (0%)	4/50 (8%)	
Total proteins	Decreased	2	0/3 (0%)	2/75 (2.7%)	0.28
Total proteins	Increased		1/3 (33.3%)	5/75 (6.7%)	
Albumins	Decreased	0	4/4 (100%)	63/76 (82.9%)	1
Globulins	Decreased	2	0/3 (0%)	1/75 (1.3%)	0.612
Globulins	Increased		2/3 (66.7%)	34/75 (45.3%)	
Urea nitrogen	Decreased	3	2/3 (66.7%)	7/74 (9.5%)	0.017*
Urea nitrogen	Increased		1/3 (33.3%)	10/74 (13.5%)	
Creatinine	Increased	2	1/4 (25%)	7/74 (9.5%)	0.346
Glucose	Increased	2	2/3 (66.7%)	41/75 (54.7%)	1
Cholesterol	Increased	3	1/3 (33.3%)	1/74 (1.4%)	0.111
Triglycerides	Decreased	2	0/3 (0%)	3/75 (4%)	1

Triglycerides	Increased		0/3 (0%)	4/75 (5.3%)	
Total bilirubin	Increased	1	1/4 (25%)	3/75 (4%)	0.191
ALP	Increased	0	1/4 (25%)	3/76 (3.9%)	0.189
ALT	Increased	4	0/4 (0%)	0/72 (0%)	
AST	Increased	1	1/4 (25%)	8/75 (10.7%)	0.39
γGT	Increased	5	0/3 (0%)	1/72 (1.4%)	1
Calcium	Decreased	2	0/4 (0%)	6/74 (8.1%)	1
Calcium	Increased		0/4 (0%)	1/74 (1.4%)	
Inorganic phosphorus	Increased	3	2/4 (50%)	8/73 (11%)	0.08
Potassium	Decreased	1	0/3 (0%)	1/76 (1.3%)	1
Potassium	Increased		0/3 (0%)	10/76 (13.2%)	
Sodium	Decreased	1	1/3 (33.3%)	15/76 (19.7%)	0.63
Sodium	Increased		0/3 (0%)	6/76 (7.9%)	
Chloride	Decreased	1	2/3 (66.7%)	27/76 (35.5%)	0.569
Chloride	Increased		0/3 (0%)	1/76 (1.3%)	

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; γGT: gamma-glutamyl transpeptidase; HCT: haematocrit

## CONCLUSIONS

1. The prevalence of FeLV infection in cats from Greece was found to be 3.9%.
2. Cats with a history of vomiting or rhinitis are significantly more likely to be FeLV-positive compared to cats without vomiting and rhinitis, respectively.
3. Infection with FIV increases the risk of infection with FeLV.
4. Neutropenic cats are significantly more likely to be FeLV-positive compared to cats with neutrophil counts within the reference intervals.
5. Cats with decreased BUN concentration are significantly more likely to be FeLV-positive compared to cats with BUN concentration within the reference intervals.
6. Cats with increased serum cholesterol or increased serum triglyceride concentration are significantly more likely to be FeLV-positive compared to cats with cholesterol and triglyceride concentrations within the reference intervals.
7. The prevalence of FIV infection in cats from Greece was found to be 9.2%.
8. Male gender, older age, and outdoor access have been identified as risk factors for FIV seropositivity.
9. Weight loss and the presence of fever, gingivostomatitis and skin lesions and/or pruritus are significantly associated with FIV-seropositivity.
10. Cats with hyperglobulinemia are more likely to be FIV-seropositive compared to cats with normal or reduced serum globulin concentrations.
11. The seroprevalence of FCoV infection in cats from Greece was found to be 12.1%.
12. Cats adopted as strays and in contact with other cats are more likely to be seropositive for FCoV.
13. The prevalence of seropositivity for *B. henselae* was found to be 35.4%.
14. Stray cats, cats with outdoor access, multiple in-contact cats and flea infestation are risk factors for *B. henselae*-seropositivity.
15. Gingivostomatitis is significantly associated with *B. henselae*-seropositivity.
16. Hyperglobulinemia is significantly associated with *B. henselae*-seropositivity.
17. Cats with an increased serum phosphorus concentration are significantly more likely to be *B. henselae*-seronegative than cats with a normal serum phosphorus concentration.

18. The molecular prevalence of *Bartonella* spp. infection in cats from Greece was found to be 2.9%.
19. Living in rural areas is a risk factor for *Bartonella* spp. PCR-positivity.
20. Non-smooth surface of the kidneys on abdominal palpation is significantly associated with PCR-positivity for *Bartonella* spp.
21. Cats with hypercalcemia are more likely to be *Bartonella* spp.-positive compared to cats with normal or reduced serum calcium concentrations.
22. The prevalence of hemoplasma species infection in cats from Greece was found to be 19%.
23. The lack of ectoparasiticide use is a risk factor for hemoplasma species infection.
24. Seropositivity for *T. gondii* has been found to be significantly associated to hemoplasma species PCR-positivity.
25. The presence of fever and ocular signs are significantly associated with PCR-positivity for hemoplasma species.
26. The prevalence of hemoplasma species PCR-positivity in clinically healthy cats is higher compared to sick cats.
27. Cats with thrombocytopenia are significantly more likely to be PCR-positive for hemoplasma species compared to cats with a normal platelet count.
28. The seroprevalence of *T. gondii* infection in cats from Greece was found to be 20.8%.
29. Older age is a risk factor for *T. gondii*-seropositivity.
30. Cats with a history of cat-bite wounds are significantly more likely to be *T. gondii*-seropositive compared to cats with no such history.
31. Cats that have not been vaccinated against calicivirus, herpesvirus-1, panleukopenia and rabies are significantly more likely to be seropositive to *T. gondii* compared to vaccinated cats.
32. The prevalence of feline hyperthyroidism among cats from Greece with an age of at least 6 years was found to be 5%.
33. The frequency of palpable goiter, depression-lethargy and dyspnea was significantly higher in hyperthyroid compared to euthyroid cats.
34. Decreased BUN concentration was more common in the hyperthyroid compared to euthyroid cats.

## PROPOSALS FOR FUTURE STUDIES

The results of this study provide important information regarding the prevalence, the risk factors, the clinical picture and the clinicopathologic abnormalities of FeLV, FIV, FCoV, *Bartonella* spp., hemoplasma species, *Toxoplasma gondii* infections and feline hyperthyroidism. Future research could:

1. Determine the prevalence of feline infectious peritonitis in cats from Greece and investigate the risk factors for the appearance of the disease.
2. Investigate the clinical and clinicopathologic relevance of the infection by the different species of *Bartonella* and of hemoplasmas in cats.
3. Investigate the mechanisms underlying the positive association between gingivostomatitis and *Bartonella* spp. infection.
4. Investigate the role of fleas and ticks as vectors for hemoplasma species.
5. Determine the prevalence of feline toxoplasmosis in Greece and assess the risk factors for the appearance of the disease.
6. Determine the risk factors for the appearance of feline hyperthyroidism in Greece and further investigate its clinical and clinicopathologic manifestations, addressing a larger number of cats and using additional examinations to confirm the diagnosis in equivocal cases.

## SUMMARY

### STUDY ON THE EPIDEMIOLOGY AND THE CLINICAL IMPORTANCE OF SELECTED INFECTIOUS, PARASITIC AND METABOLIC DISEASES IN CATS FROM GREECE

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Doctoral Thesis 2021

Infectious and parasitic diseases are common in all mammalian species, including domestic cats (*Felis catus*), who are becoming increasingly popular pets in Greece. Although several studies have investigated the prevalence of certain infectious and parasitic diseases in dogs, little is known about the prevalence and epidemiology of specific infectious agents in cats from Greece. This lack of data represents a major drawback for accurate and effective diagnosis, clinical management, and prevention of the diseases caused by these agents.

Vector-borne diseases, not only impact the health of domestic cats, but they are often of zoonotic importance. Since fleas, ticks and mosquitos are common in Greece, vector-borne agents such as *Bartonella* spp. and hemoplasma species, may also be common. Other infectious and parasitic agents such as *Toxoplasma gondii*, feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline coronavirus (FCoV) are also known to be present in Greece but reports on their prevalence, epidemiology, and clinical manifestations are scarce or absent.

Feline hyperthyroidism is worldwide the most common endocrinopathy in cats and its prevalence is increasing over time. No data on the prevalence of feline hyperthyroidism in Greece are available.

The aims of this study are to: a) determine the seroprevalence for FeLV, FIV, FCoV, *B. henselae* and *T. gondii* and the molecular prevalence of *Bartonella* spp. and *Mycoplasma* spp. infections; b) determine the prevalence of feline hyperthyroidism; c) investigate risk factors for FeLV, FIV, FCoV, *B. henselae* and *T. gondii* seropositivity; d) investigate risk factors for *Bartonella* spp. and *Mycoplasma* spp. PCR-positivity; e) determine the clinical signs, hematological and biochemical

findings associated with FeLV, FIV, FCoV, *B. henselae* and *T. gondii* seropositivity; f) determine the clinical signs, hematological and biochemical findings associated with PCR-positivity for *Bartonella* spp. and *Mycoplasma* spp.; g) investigate possible associations between seropositivity for *B. henselae*, PCR-positivity for *Bartonella* spp. or PCR-positivity for *Mycoplasma* spp. and seropositivity for other infectious agents, including *T. gondii*, FCoV, FeLV and FIV; h) to compare the clinical signs, hematological and serum biochemical findings between hyperthyroid and non-hyperthyroid cats.

A total of 547 clinically healthy and sick cats were prospectively enrolled in the study. The age of the cats ranged from 6 weeks to 17 years, they lived in Attica, Crete, Thessaly, and Macedonia and were client-owned, stray, or cattery cats. Four subgroups of 435, 453, 452 and 457 cats were selected to investigate the epidemiology and the clinical importance of infection with FeLV/FIV, FCoV, *Bartonella* spp./hemoplasma species, and *T. gondii*, respectively, while a fifth subgroup of 80 cats was selected for the investigation of the prevalence of hyperthyroidism. After history and physical examination, blood sampling was performed. Laboratory examinations included: a) hematology; b) serum biochemistry; c) serology for the detection of FeLV antigen and anti-FIV IgG antibodies using a commercial in-house ELISA test kit; d) serology (IFAT) for the detection of anti-FCoV, anti-*B. henselae* and anti-*T. gondii* IgG antibodies; e) blood PCR for detection of *Bartonella* spp. and hemoplasma species DNA; f) solid-phase chemiluminescent competitive assays for measurement of serum total T<sub>4</sub> and free T<sub>4</sub> concentration.

The seroprevalence of FeLV, FIV, FCoV, *B. henselae* and *T. gondii* infection was found to be 3.9%, 9.2%, 12.1%, 35.4% and 20.8%, respectively. The molecular prevalence of *Bartonella* spp. and hemoplasma species infection was found to be 2.9% and 19%, respectively. Finally, the prevalence of feline hyperthyroidism was found to be 5%.

Independent risk factors for FeLV seropositivity included vomiting, rhinitis, infection with FIV, neutropenia, decreased BUN and increased serum cholesterol and triglyceride concentrations.

Male gender, older age, outdoor access, weight loss, fever, gingivostomatitis, presence of skin lesions and/or pruritus, and hyperglobulinemia were independent risk factors for FIV seropositivity.

Cats adopted as stray cats and being in contact with other cats were at increased risk for FCoV seropositivity.

Independent risk factors for *B. henselae* seropositivity included being a stray cat, having outdoor access, having multiple in-contact cats, history of flea infestation, gingivostomatitis, hyperglobulinemia, and increased serum inorganic phosphorus concentration. Living in rural areas, non-smooth kidney surface on abdominal palpation and hypercalcemia were risk factor for *Bartonella* spp. PCR-positivity.

Independent risk factors for positive blood PCR for hemoplasma species included the lack of use ectoparasiticides, seropositivity for *T. gondii*, fever, presence of ocular signs, thrombocytopenia, and being clinically healthy cat.

Older age, history of cat-bite wounds, and no vaccination against calicivirus, herpesvirus-1, panleukopenia and rabies were risk factors for *T. gondii*-seropositivity.

The frequency of palpable goitre, depression-lethargy, dyspnea, and decreased BUN concentration was significantly higher in hyperthyroid compared to euthyroid cats.

In conclusion, the results of this study indicate a relative high prevalence of FeLV, FIV, FCoV, *Bartonella* spp., hemoplasma species and *T. gondii* seropositivity and/or PCR positivity in cats in Greece. Various factors related to the signalment and history, clinical examination findings, and laboratory abnormalities were found to be associated with the exposure and/or infection with these organisms. Thus, it is necessary to establish optimal strategies for prevention and management of these infections, taking into consideration the high-risk groups of cats identified in this study. Finally, hyperthyroidism is relatively common in cats in Greece, from the age of 6 years and above and should be considered especially in cats with palpable goiter, depression-lethargy, dyspnea and/or decreased BUN concentration.



## ΠΕΡΙΛΗΨΗ

### ΜΕΛΕΤΗ ΤΗΣ ΕΠΙΔΗΜΙΟΛΟΓΙΑΣ ΚΑΙ ΤΗΣ ΚΛΙΝΙΚΗΣ ΣΗΜΑΣΙΑΣ ΕΠΙΛΕΓΜΕΝΩΝ ΛΟΙΜΩΔΩΝ, ΠΑΡΑΣΙΤΙΚΩΝ ΚΑΙ ΜΕΤΑΒΟΛΙΚΩΝ ΝΟΣΗΜΑΤΩΝ ΤΗΣ ΓΑΤΑΣ ΣΤΗΝ ΕΛΛΑΔΑ

Κασσιόπη-Χριστίνα Γ. Κοκκινάκη

Διδακτορική Διατριβή 2021

Όλα τα ζωικά είδη μπορεί να εκτεθούν και να νοσήσουν από μεγάλο αριθμό μικροοργανισμών, συμπεριλαμβανομένης της γάτας (*Felis catus*), ενός είδους που απαντάται όλο και συχνότερα στην καθημερινή κλινική πράξη. Παρ' όλο που στην Ελλάδα υπάρχουν αρκετές έρευνες για τη συχνότητα και τους παράγοντες επικινδυνότητας για την εμφάνιση λοιμωδών και παρασιτικών νοσημάτων στο σκύλο, οι αντίστοιχες μελέτες για τη γάτα είναι λιγιστές, γεγονός που αποτελεί τροχοπέδη για τον σχεδιασμό κατάλληλων διαγνωστικών, θεραπευτικών και προληπτικών παρεμβάσεων, τόσο στις οικόσιτες γάτες όσο σε εκείνες που ζουν σε εκτροφεία και καταφύγια ή είναι αδέσποτες. Επιπλέον, στη χώρα μας δεν υπάρχει δημοσιευμένη μελέτη για τη συχνότητα του υπερθυρεοειδισμού, που διεθνώς θεωρείται η συχνότερη ενδοκρινοπάθεια της γάτας.

Τα λοιμώδη νοσήματα της γάτας που μεταδίδονται με ενδιάμεσους ξενιστές έχουν παγκόσμια εξάπλωση, ενώ συχνά προσβάλλουν και τον άνθρωπο. Οι βιολογικοί ή μηχανικοί μεταδότες είναι αρθρόποδα, όπως ψύλλοι, κρότωνες και κουνούπια, τα οποία αφθονούν στην Ελλάδα και είναι ικανά να μεταδώσουν διάφορους λοιμογόνους παράγοντες, όπως για παράδειγμα η *Bartonella* spp. και το *Mycoplasma* spp. Ορισμένοι άλλοι μικροοργανισμοί, όπως είναι ο ιός της λευχαιμίας (FeLV), ο ιός της επίκτητης ανοσοανεπάρκειας (FIV) και ο κορονοϊός (FCoV) της γάτας, είναι γνωστό ότι υπάρχουν στην Ελλάδα, αλλά η επιδημιολογική και η κλινική τους σημασία παραμένει αδιευκρίνιστη. Τέλος, σε επιδημιολογικές μελέτες, από διάφορες χώρες, έχει βρεθεί ότι το ποσοστό των γατών και των ανθρώπων που είναι ορολογικά θετικοί για το *Toxoplasma gondii* μπορεί να ξεπερνάει το 30%, γεγονός που καταδεικνύει την

αναγκαιότητα σύγχρονων δεδομένων για τη συχνότητα της τοξοπλάσμωσης της γάτας στην Ελλάδα και των παραγόντων επικινδυνότητας.

Οι στόχοι της μελέτης αυτής ήταν: α) η διερεύνηση της συχνότητας της οροθετικότητας της γάτας στην Ελλάδα έναντι των FeLV, FIV, FCoV, *B. henselae* και *T. gondii* και της συχνότητας μόλυνσης από *Bartonella* spp. και *Mycoplasma* spp., β) ο προσδιορισμός της συχνότητας του υπερθυρεοειδισμού, γ) η διερεύνηση των παραγόντων επικινδυνότητας για την οροθετικότητα έναντι των FeLV, FIV, FCoV, *B. henselae* και *T. gondii*, δ) η διερεύνηση των παραγόντων επικινδυνότητας για τη μόλυνση από *Bartonella* spp. και *Mycoplasma* spp., ε) η διερεύνηση των συμπτωμάτων, των αιματολογικών και βιοχημικών διαταραχών που σχετίζονται με την οροθετικότητα έναντι των FeLV, FIV, FCoV, *B. henselae* και *T. gondii*, στ) η διερεύνηση των συμπτωμάτων, των αιματολογικών και βιοχημικών διαταραχών που σχετίζονται με την μόλυνση από *Bartonella* spp. και *Mycoplasma* spp., ζ) η διερεύνηση πιθανών συσχετισμών μεταξύ της οροθετικότητας για *B. henselae*, της μόλυνσης από *Bartonella* spp. ή της μόλυνσης από *Mycoplasma* spp. και της οροθετικότητας για FeLV, FIV, FCoV, και *T. gondii*, η) η σύγκριση της κλινικής εικόνας και των αποτελεσμάτων της γενικής εξέτασης του αίματος και των βιοχημικών εξετάσεων μεταξύ των υπερθυρεοειδικών και ευθυρεοειδικών γατών.

Στην μελέτη περιλήφθηκαν συνολικά 547 κλινικά υγιείς και άρρωστες γάτες. Η ηλικία τους κυμαινόταν μεταξύ 6 εβδομάδων και 17 ετών και ζούσαν στην Αττική, την Κρήτη, τη Θεσσαλία και τη Μακεδονία. Οι γάτες της μελέτης ήταν δεσποζόμενες, αδέσποτες ή ζούσαν σε εκτροφές. Από τις 547 γάτες, επιλέχθηκαν οι 435, 453, 452 και 457 για τη μελέτη της επιδημιολογίας και της κλινικής σημασίας της μόλυνσης από FeLV/FIV, FCoV, *Bartonella* spp./*Mycoplasma* spp. και *T. gondii* αντίστοιχα, ενώ στη μελέτη του υπερθυρεοειδισμού χρησιμοποιήθηκαν 80 γάτες ηλικίας  $\geq 6$  ετών. Μετά την λήψη του ιστορικού και την κλινική εξέταση ακολούθησε η λήψη ολικού αίματος. Οι εξετάσεις που πραγματοποιήθηκαν ήταν: α) γενική εξέταση αίματος, β) βιοχημικές εξετάσεις στον ορό αίματος, γ) ορολογική εξέταση για την ανίχνευση του αντιγόνου του FeLV και των αντισωμάτων έναντι του FIV, δ) ορολογικές εξετάσεις (IFAT) για την ανίχνευση IgG αντισωμάτων έναντι της *B. henselae*, του *T. gondii* και του FCoV, ε) PCR από το αίμα για την ανίχνευση του DNA των *Bartonella* spp. και *Mycoplasma* spp., και στ) προσδιορισμός της συγκέντρωσης της ολικής T<sub>4</sub> και της ελεύθερης T<sub>4</sub>.

Η συχνότητα της οροθετικότητας για FeLV, FIV, FCoV, *B. henselae* και *T. gondii* ήταν 3,9%, 9,2%, 12,1%, 35,4% and 20,8%, αντίστοιχα, η συχνότητα της μόλυνσης από *Bartonella* spp. και *Mycoplasma* spp. ήταν 2,9% και 19%, αντίστοιχα και η συχνότητα του υπερθυρεοειδισμού ήταν 5%.

Οι παράγοντες επικινδυνότητας για τη μόλυνση από FeLV ήταν η διαπίστωση εμέτου ή ρινίτιδας, η ταυτόχρονη μόλυνση από FIV, η ουδετεροπενία, η μειωμένη συγκέντρωση ουρικού αζώτου (BUN) στον ορό αίματος και η αυξημένη συγκέντρωση χολοστερόλης και τριγλυκεριδίων.

Οι παράγοντες επικινδυνότητας για την οροθετικότητα στον FIV ήταν το αρσενικό φύλο, η μεγαλύτερη ηλικία, η διαμονή σε εξωτερικούς χώρους, η απώλεια βάρους, ο πυρετός, η διαπίστωση δερματικών αλλοιώσεων ή/και κνησμού και η υπερσφαιριναιμία.

Οι παράγοντες επικινδυνότητας για την οροθετικότητα στον FCoV ήταν η υιοθέτηση προηγουμένως αδέσποτης γάτας και η επαφή με άλλες γάτες.

Παράγοντες επικινδυνότητας για την οροθετικότητα στην *B. henselae* ήταν το να είναι η γάτα αδέσποτη, να ζει σε εξωτερικούς χώρους, να έρχεται σε επαφή με αυξημένο αριθμό γατών, να έχει ιστορικό παρασίτωσης από ψύλλους, να εμφανίζει ουλοστοματίτιδα ή υπερσφαιριναιμία και να μην εμφανίζει υπερφωσφαταιμία.

Η διαβίωση σε αγροτικές περιοχές, η ανώμαλη επιφάνεια των νεφρών κατά την ψηλάφησης της κοιλίας και η υπερασβεστιαίμια ήταν οι παράγοντες επικινδυνότητας για τη μόλυνση από *Bartonella* spp.

Στους παράγοντες επικινδυνότητας για τη μόλυνση από *Mycoplasma* spp. περιλαμβάνονται η μη χρήση εξωπαρασιτοκτόνων, η οροθετικότητα στο *T. gondii*, ο πυρετός, οι αλλοιώσεις από τους οφθαλμούς, η μη διαπίστωση παθολογικών ευρημάτων κατά την κλινική εξέταση και η θρομβοκυτταροπενία.

Οι παράγοντες επικινδυνότητας για την οροθετικότητα στο *T. gondii* ήταν η αυξημένη ηλικία, τα δαγκώματα από άλλες γάτες και ο μη εμβολιασμός έναντι του καλικοϊού, του ερπητοϊού, της πανλευκοπενίας και της λύσσας.

Η συχνότητα βρογχοκήλης, κατάπτωσης ή ληθαργικότητας, δύσπνοιας και μειωμένης συγκέντρωσης BUN ήταν μεγαλύτερη στις υπερθυρεοειδικές γάτες σε σχέση με τις ευθυρεοειδικές.

Συμπερασματικά, από τα αποτελέσματα της μελέτης προκύπτει ότι η συχνότητα οροθετικότητας ή/και μόλυνσης της γάτας στην Ελλάδα από FeLV, FIV, FCoV, *Bartonella* spp., *Mycoplasma* spp. και *T. gondii* είναι σχετικά μεγάλη και

σχετίζεται με διάφορα συμπτώματα και εργαστηριακές διαταραχές, ανάλογα με το μικροοργανισμό. Για το λόγο αυτό, είναι απαραίτητο να σχεδιαστούν κατάλληλα διαγνωστικά, θεραπευτικά και προληπτικά μέτρα, που θα λαμβάνουν υπόψη του παράγοντες αυξημένου κινδύνου. Τέλος, ο υπερθυρεοειδισμός της γάτας φαίνεται να είναι συχνός στην Ελλάδα στις γάτες ηλικίας  $\geq 6$  ετών και πρέπει να ελέγχεται, ιδιαίτερα στις μεσήλικες και υπερήλικες γάτες με βρογχοκήλη, κατάπτωση ή ληθαργικότητα και μειωμένη συγκέντρωση BUN.